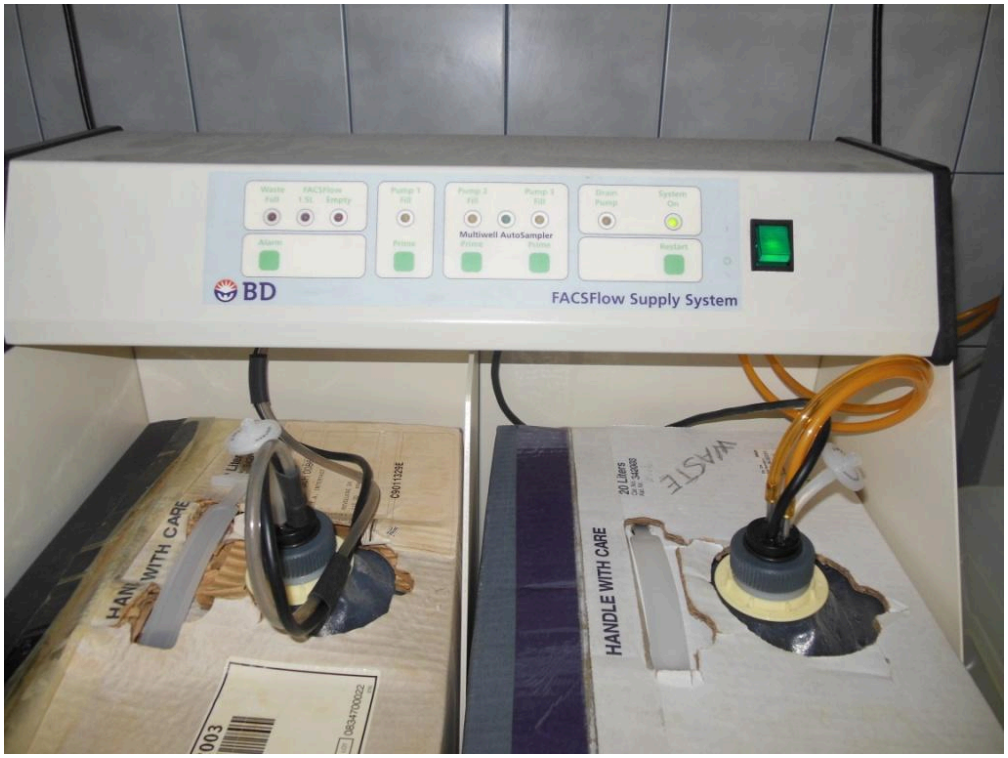
LSR II guide

Ensure that you signed up for the instrument use in the on-line calendar for 24 hours / 7 days per week including weekends.

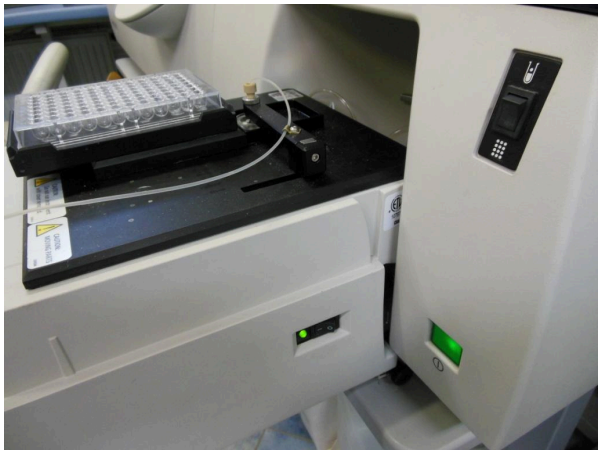
Startup (takes 15 min)

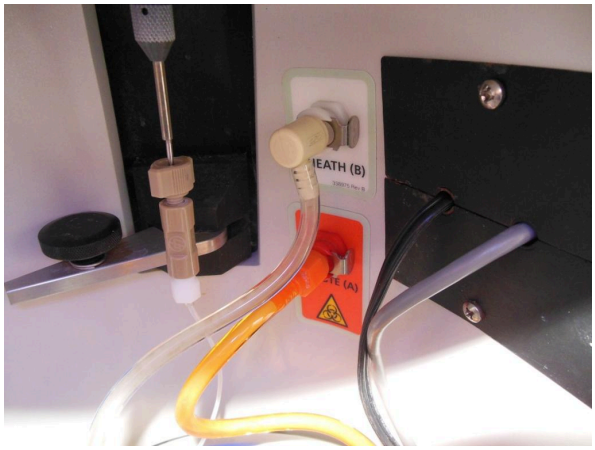
- **Switch ON the LSRII instrument** (green button). It takes approximately 15 minutes to fully stabilize the operation of all lasers!!!
- After 10 seconds switch ON the computer
- **Prime fluidics before each run**
    - change tube/plate switch on the LSRII to tube
    - Put the tube away-so there is only the needle visible
    - Press PRIME button and after 3 seconds immediately press RUN button - you should see small droplets falling from needle
    - Repeat these two steps in total 2 times
    - Place instrument to STANDBY until you are ready to run the experiment and install tube with cca 2ml of dH2O

- Log into Windows
- Start DiVa
- After switching ON LSR II and always before starting your run (if the system is ON): **Check the shealth and waste containers in FACS Flow Supply System (FFSS) and check if the green button is lightning:**
    - **sheath reservoir** must be filled above the mark. If not, it is necessary to refill it with 10 L of sheath fluid (made of 1 L of 10x PBS stock followed by 9 L of distilled water). Make sure tank is tightly closed after filling.
    - **waste reservoir** must be below the mark. If not, Empty if by discarding its contents into sink and add 50 mL bleach (SAVO) into the empty waste tank before returning it to the machine.
    - **press RESTART button on FFSS control panel !!!**
    - ***Always make sure the shealth container does not get empty!!!!!***



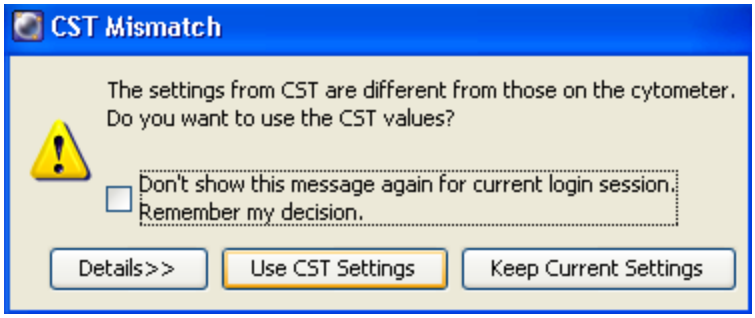
- I**f you are going to use HTS**:
    - switch ON HTS loader (black button with green light)
    - connect the capillary with brown connector onto the needle
        - **hold it via the brown mid-piece and <u>not the white screw</u> !**
        - **squeeze it upwards until you feel the needle touching the connector**
        - **tighten the brown screw such that the connector does not move on the stainless needle**
    - change the tube => plate switch on the front panel of LSR
    - check if small magnet is in place on the front edge of HTS (covered by blue tape), otherwise HTS announces the error attempting to operate without the cover
    - **<u>remove the lid from plate</u>**





Run

- Start FACSDiva software and log into it. Wait for cytometer to connect. If cytometer does not connect, switch it OFF, wait 10 seconds and switch ON again. Reset the computer 10 seconds after the cytometer and start DIVA.
- if this window appears, click on Use CST Settings



- If you run the same type of experiment before (can be saved as your blank experiment), open that experiment, duplicate it without data (Ctrl+D) and rename.
- Otherwise, create a new experiment ("Blank Experiment with Sample Tube") or use a template from the USER tab.
- Do not collect unnecessary or unwanted parameters to your data files (to prevent collection of unnecessary parameters you must delete these parametres from Instrument settings/Parametres of your experiment). Do not collect pulse height or pulse width parameters if there isn´t specific reason for their collection.
- There are 2 modes of cytometer settings available: Cytometer settings unique for each experiment (user defined) or Application settings which keep the system calibrated over long periods of time (defined by CS&T Performance check) - will be updated later
- Turn to RUN mode (cytometer front panel) and acquire samples ideally on LOW or MEDIUM flow rates where you achieve the best date quality. There are differences

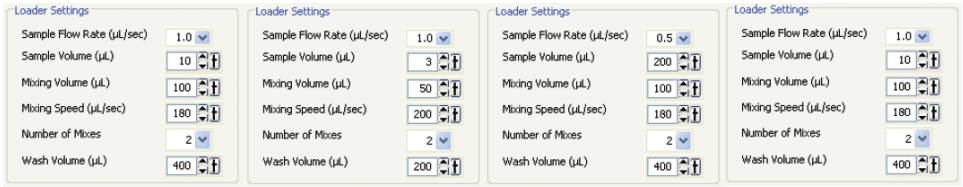
between tube and plate (HTS loader) acquisition flow rates:

- tube operated from the front panel on the cytometer
  - LOW = 12uL/min
  - MED = 30uL/min
  - HIGH = 60uL/min
  - with the potentiometer knob multiplicating these values 0,5 - 2x (left to right)
- HTS loader operated from menu Plate Loader Settings
  - 0,5 - 3uL/sec ~ 30 - 180uL/min

HTS Loader Settings Overview

HTS loader settings are specified under the Setup tab of the Plate window. Ensure that the loader settings are appropriate for your sample volume, sample concentration, and the specified events to record.

Default Loader Settings



Specimen wells using Standard Throughput mode | Specimen wells using High Throughput mode | Setup Control wells | Compensation Control wells

Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in µL per second) that is delivered to the flow cell. Select a rate between 0.5 and 3.0 in increments of 0.5 µL per second.	The larger the value entered, the shorter the plate running time, but this increases the sample core, causing more variation of data.
Sample Volume	Amount of sample (in µL) aspirated from the well and delivered to the flow cell. Select a volume between 2 and 200 µL.	For High Throughput mode, the system aspirates a set amount of 22 µL of sample, but records data for a volume between 2 and 10 µL. For Standard Throughput mode, the system aspirates the sample volume amount plus 20 µL. This value does not include the system default volume or the plate-dependent dead volume.
Mixing Volume	Amount of sample (in µL) aspirated and dispensed from the well to resuspend the particles.	To avoid introducing bubbles into the fluidics, this value should be half the total well volume.
Mixing Speed	Rate (in µL per second) that the mixing volume sample is aspirated and dispensed.	The faster the rate, the more likely that cell shearing occurs, especially for delicate cells. A faster rate can introduce bubbles in the sample delivered to the cytometer and compromise the separator bubble.
Number of Mixes	The number of times the mixing volume sample is aspirated and dispensed at the mixing speed. Select a number between 0 and 5 mixes.	The larger the number, the longer the plate running time.
Wash Volume	Amount of sheath fluid (in µL) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 µL.	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.

Shutdown / Finished (takes 15 min)

- Export your data (FSC 2.0 or FSC 3.0) or the complete Experiment (can be imported in another DiVa computer including Worksheet, Gating, Cytometer settings and Statistics)
- Delete your experiment if it is not necessary to keep it in the cytometer computer. **The database must be kept as small as possible! Each user has maximum space 1GB.**
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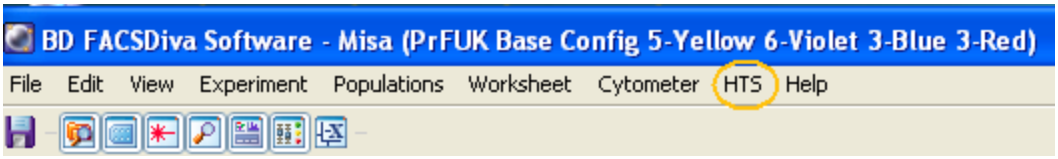
- If you have measured samples from **tubes** wash the instrument by putting it to HIGH mode and run tube with 10% bleach (Savo) for at least 3 min, then run tube with dH2O for 3 min. At the end, install the tube with no more than 1cm of deionized water and put the instrument to STANDBY mode. (The amount of water in tube is critical to prevent the moisturing of air filters inside the instrument).
- If you have used **HTS** for measurement, perform the HTS Daily clean
    1. prepare and install cleaning 96-well plate. Put 10% bleach (Savo) to wells A1-A4, put dH2O to wells B1-B4.
    2. In DIVA software > HTS menu > click on Daily clean and follow the instructions.



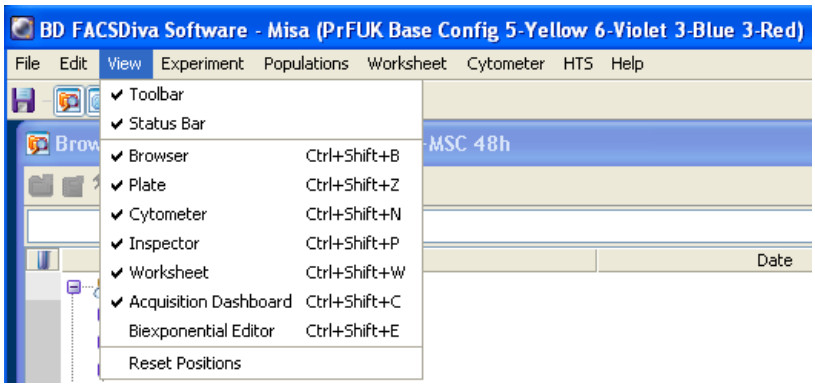
- Log out from software!
- **Check the LSRII webcalendar. If there is another user scheduled to use instrument in less then three hours, put the instrument to STANBY and leave the instrument running.**
- If you are the **last user of the day**,TURN OFF LSRII unit and shut down computer.
    - ***Do not allow LSRII to run overnight!***

Troubleshooting

Always make sure the shealth container does not get empty!!!!!
Always filter clumpy samples !!
Do not touch lasers !!

1. Computer can´t connect to cytometer/ problems with DIVA

- ➢ Restart Diva software.
- ➢ Display of working windows.



2. No events

- ➢ Laser(s) not turned on.
- ➢ Fluidics control not set to "RUN".
- ➢ Tube is cracked.
- ➢ Check level in sheath tank.
- ➢ HTS loader not connected properly - check switches and capilares.
- ➢ No cells in sample.
- ➢ Clumpy sample - filter it.
- ➢ Blocked SIP or flow cell - remove sample, and press PRIME. Run 3 min 100% bleach on HIGH speed, followed by 3 min dH2O.
- ➢ Perform daily clean/clean as it is described in Shutdown
- ➢ Waste not venting - check waste line for flow.
- ➢ Air bubbles in sheath filter - purge filter.

3. Sample runs for a few seconds then stops

- ➢ Clumpy sample - filter it.
- ➢ Blocked SIP or flow cell - Remove sample, and press PRIME. Run 3 min 100% bleach on HIGH speed, followed by 3 min dH2O
- ➢ Waste not venting - check waste line.
- ➢ Air bubbles in sheath filter - purge filter.

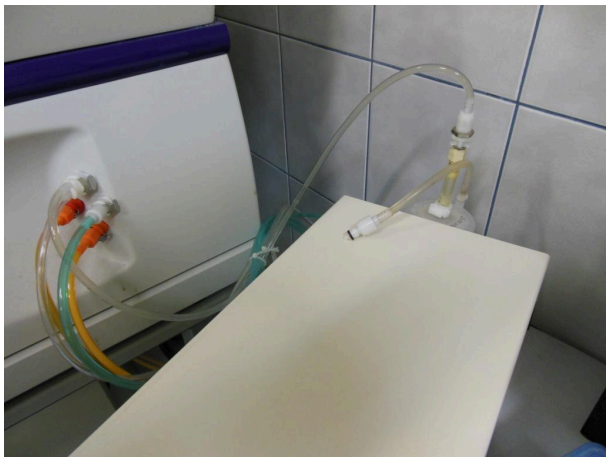
4. Very slow sample rate

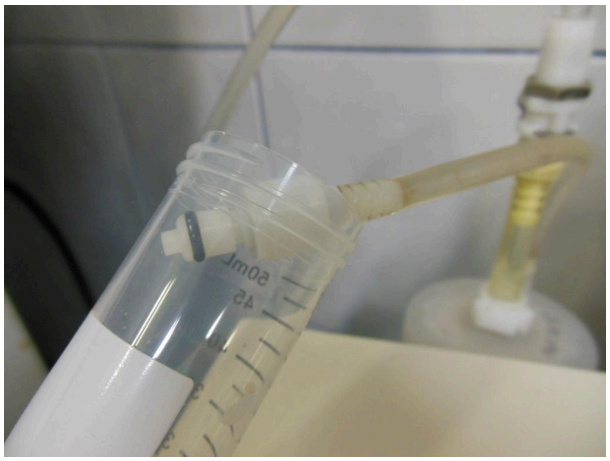
- ➢ Sample very diluted.
- ➢ Fluidics set to LOW.

5. Detection of residual signal

- ➢ Run 100% bleach or 70% ethanol for 5 minutes followed by three changes of dH2O for 1 minute each.

Purging bubbles from sheath filter : if the user has any problems with fluidics, check shealth reservoir and this shealth filter. The filter must not contain any air bubbles. If there are some, try to deaerate filter by pressing the stopper on the tubbing attached to the filter and drain the air bubbles (put some vessel below the tubing please)





<u>If you dont know what to do, please, stop measuring, press STANDBY button and call support:</u>

Michaela Hájková: 1755 (721 625 886)
Barbora Pavlů: 1795 (737 558 162)
Jana Pilátová: 608 043 910
Karel Drbal: 606 037 233

pro operátory:

6. Sample still not run

- Air bubbles in sheath filter - purge filter.

7. no PE signal: check yellow laser (restart LSRII)

8. Potentially biohazardous samples: Run TriGene (1:50 or 1:100) for 5 minutes followed by three changes of DI water for 1 minute each.

Jana časem dodá návod na kalibraci (popis + prinscreen)
Já dodělám popsaný printscreen DIVY
Bára přeloží návod do čj (pak dáme českou část návodu týkající se HTS a daily clean Janě Dvořákové)