Lyric

Operation Protokoll BD FACS Lyric

FCF-Berg

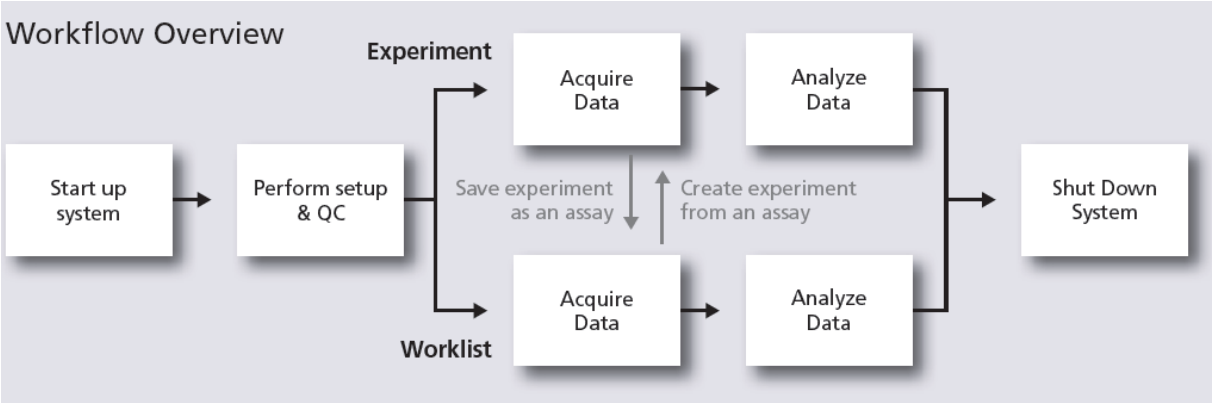
Tuebingen University

Department of Internal Medicine II

Index **Page**

With changed Sample Settings (FSC, SSC, Voltages, Flow Rate,.. shoes a star behind the Tube Properties

Setup3; Measure with your own compensation



Overview about possible modifications from the Lyse/Wash Settings

Workflow	Scenario	What is required?
Default (Lyse Wash)	Use when no adjustments need to be made to PMTVs and compensation values.	No action required
Custom 1 (Modify Lyse Wash)	Use when adjustments to PMTVs, including FSC or SSC, are necessary, but the experiment will not be repeated.	Adjust PMTVs as desired
Custom 2 (Modify Lyse Wash and Save)	Use when adjustments to PMTVs, inclusing FSC or SSC, are necessary, and the experiment will be repeated.	Create Tube Settings
Custom 3 (User-Defined Reference Settings)	Use when customized PMTVs and compensation values are necessary or if fluorochromes that do not exist in the spillover matrix are used.	Create Reference Settings
Custom 4 (Save Modified Reference Settings)	Use when existing Reference Settings require adjustments, and the experiment will be repeated.	Save modified Reference Settings

Details about the measurement

Default (Lyse Wash)	Custom 1 (Modify Lyse Wash)	Custom 2 (Modify Lyse Wash and Save)	Custom 3 (User-Defined Reference Settings)	Custom 4 (Save Modified Reference Settings)
Create tube	Create tube	Create tube	Create tube	Select tube
	Optimize PMTVs	Optimize PMTVs	Optimize PMTVs	Adjust compensation
		Create Tube Settings	Create user-defined Reference Settings by acquiring single-color compensation controls	Save Modified Reference Settings
Acquire data	Acquire data	Acquire data	Acquire data	Acquire data

Make sure the following actions have been done before running your samples

- check if the FACS Flow tank is filled and the waste container emptied
- check if the cup of the universal loader is closed
- the lasers need 20 minutes to warm up

1. Start up the system

- turn on the power of the BD FACSLyric™ by pressing the power button on the right hand side
- start up the computer and peripherals (monitor, printer etc.)
- lasers need 20 minutes to warm up before starting any acquisition work
- log in to the computer:

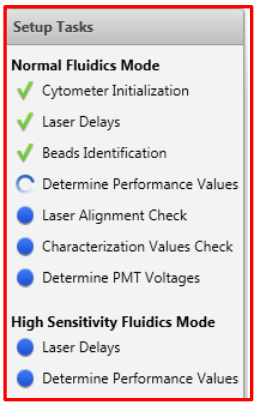
  Administrator: BDIS#1
- enter your username and password for PPMS
- start BD FACSuite™ Research Software by double click and login
- enter your username and password, click **OK**
- check the instrument status: Connected, Fluidics have to be marked green, remaining laser warm up time

2. Start the daily clean

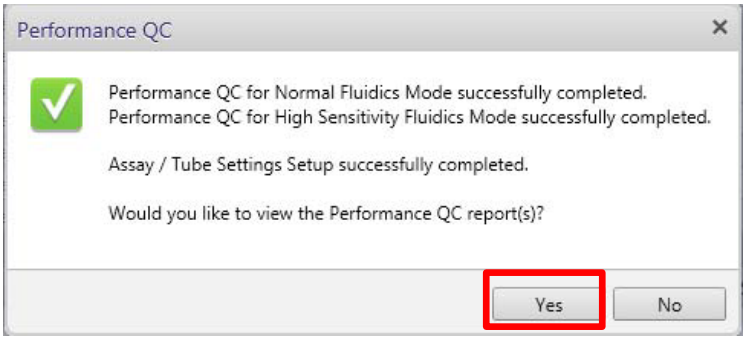
- Perform **3 times** the SIT-flush**--- Cytometer ---- Fluidics ---- SIT-Flush**
- Perform **2 times** the Daily Clean **---Cytometer ---- Daily Clean**

3. Daily Performance Quality control (QC)

- prepare the CS&T beads (shake the beads and use 2 drops with 500 µl Flow Sheat)
- navigator ---- **Setup & QC**
- verify that **Performance QC** is the selected task
- check if the correct CS&T beads lot ID is selected
- click **Start**
- mix the prepared CS&T beads, load the tube at the SIP port to start



- unload the tube with CS&T beads if the performance is done and replace a tube containing DI water



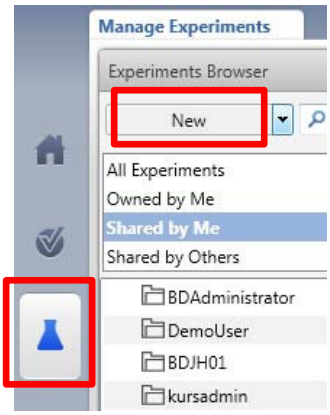
The performance QC report for normal and high sensitivity mode is displayed. To view the report press **Yes** or **No** to close the performance QC window.

4. Experiment Setup

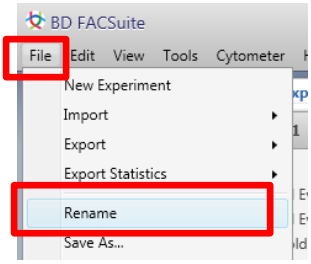
Creating a new experiment the 1st time without tube or reference settings ever being done!

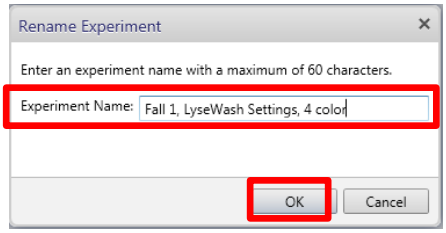
4.1 Create a New Experiment

- go to **Manage Experiments** in the workspace
- click **New** in the experiment browser

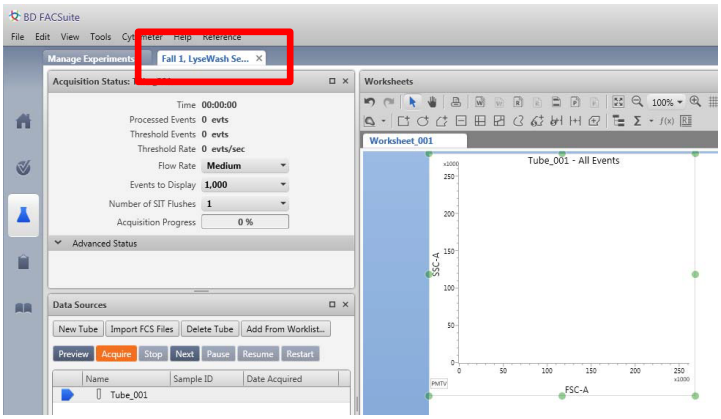


- rename the experiment **File --- Rename --- Experiment name**
- click **OK**



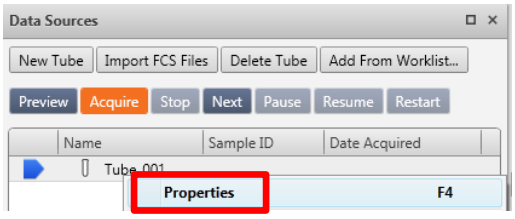


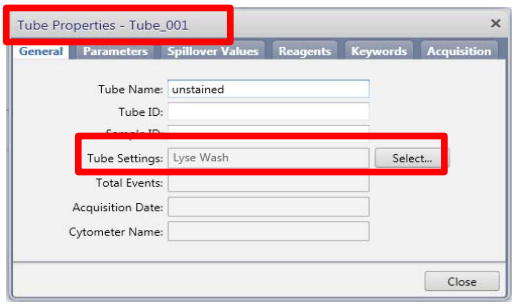
- a new experiment opens
- it includes a tube and a dot plot with FSC and SSC



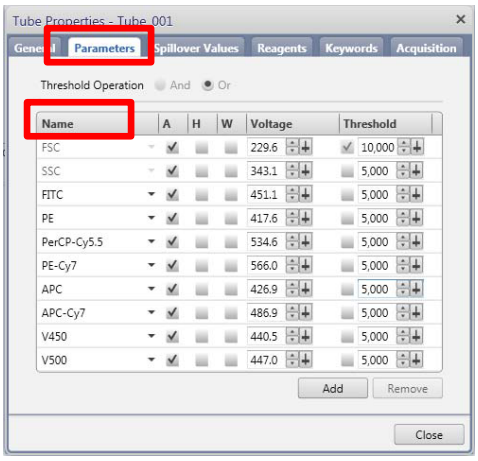
4.2 Delete or Add Parameters

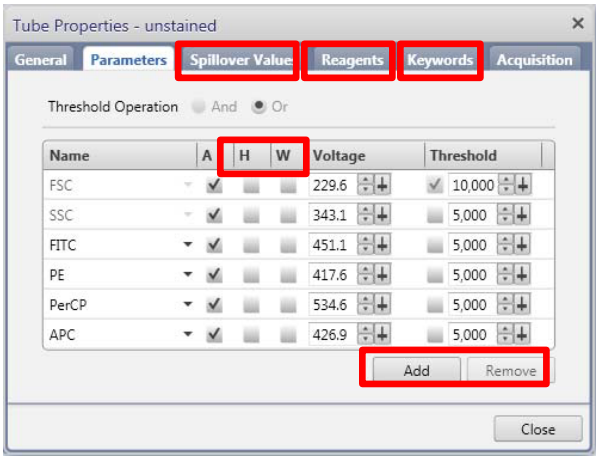
- highlight the empty tube, right click **Properties --- General,** rename the tube
- tube properties gives you also the possibility to change the tube settings





- select **Parameters**
- remove or add parameters
- select Hight and Width for FSC and SSC

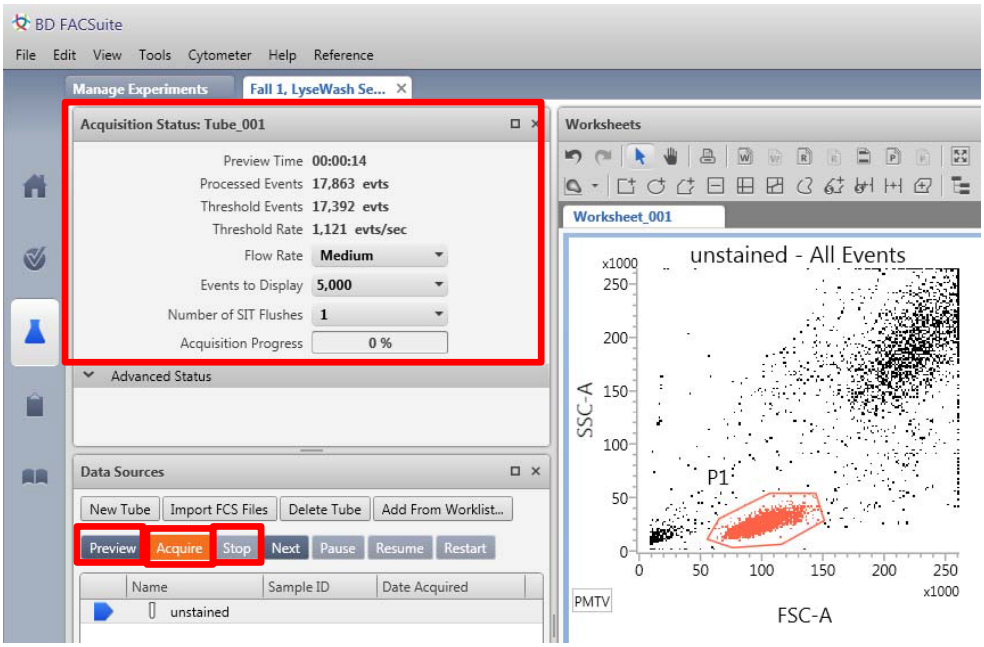




Spillover values: there are already compensation values available for the Lyse/Wash settings
FITC, PE, PerCp, PerCp-Cy5.5, PE-Cy7, APC, APC.Cy7, APC-H7, V500-C, BV605 you can also Add Flurochromes **(If you Add Flurochromes or use other parameters you have to create your own/new compensation)**
To calculate the compensation correctly, **at least 3 fluorescence parameters** must be selected!

Reagents: name the antibodies used in your experiments via the drop-down list or wright them down manually
Keywords: if you have any additional information
Acquisition: set stop criteria, gate specifications and events, to activate the stop criteria you have to click on **Apply Rules**

4.3 Acquisition Window



Preview: events are displayed but not saved

Acquire: events are recorded
Stop: stops the measurement
Next: switch to the next tube

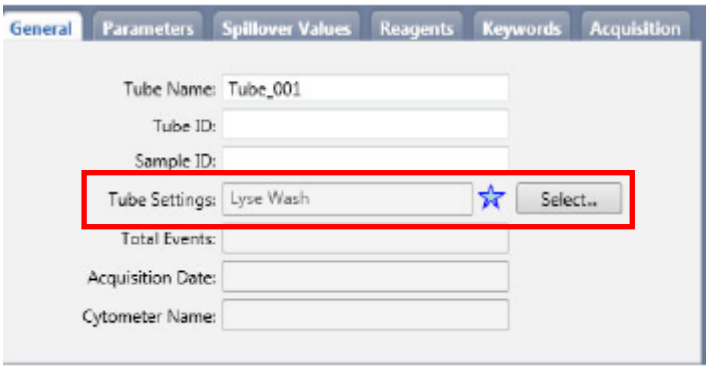
Acquisition Status:
Flow Rate: low 12µl/min, medium 60µl/min, high 120µl/min
Events to Display: numbers of events displayed in the acquisition window
Numbers of SIT-Flushes: how often the needle is cleaned after sample acquisition

The FACS Suite software has predefined tube settings for Lyse/Wash and No Lyse/No Wash as well as compensation values for both. If these tube settings are changed, e.g. flow rate, voltages of FSC/SSC, number of SIT-Flushes or fluorochomes, new tube settings must be created!

➢ go to **Measuring with own Tube Settings**

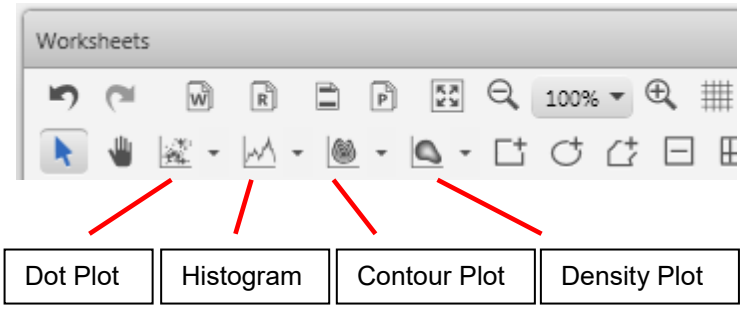
To check if the tube settings are changed check:

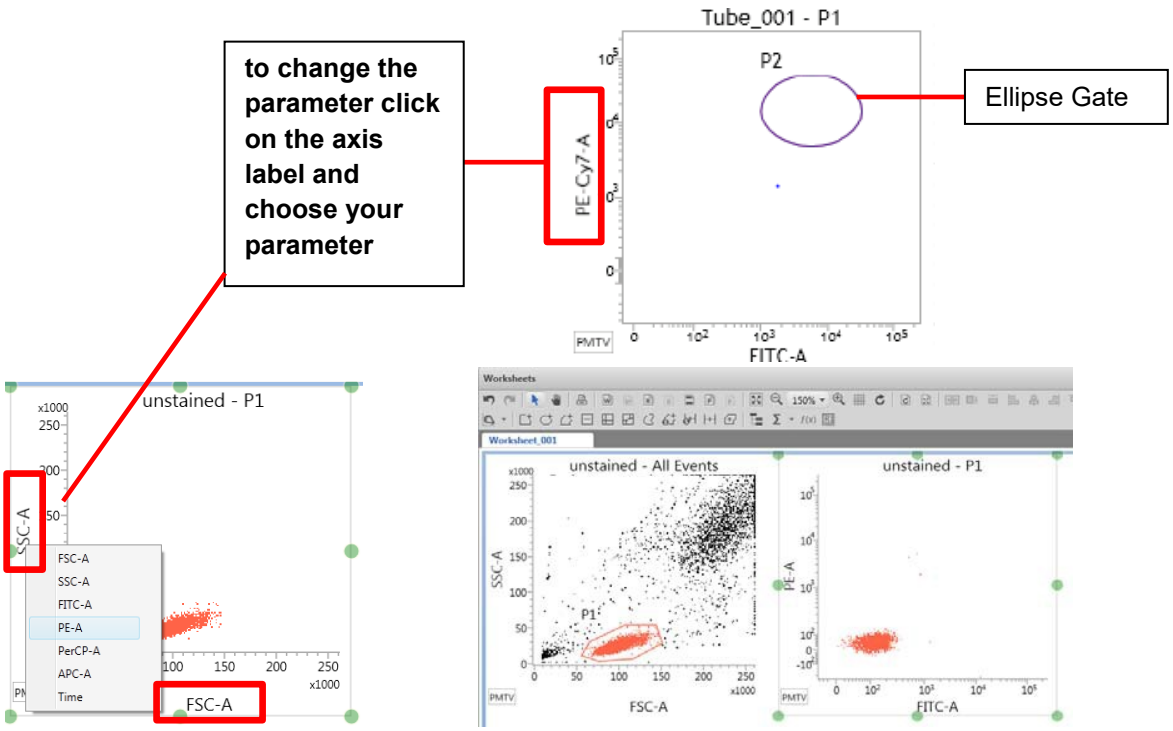
- **Tube --- Properties --- General --- Tubes settings**



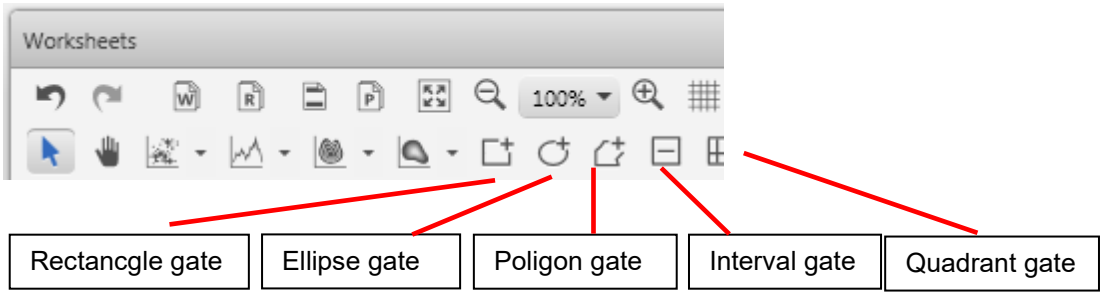
4.4 Plot creation and Gating

- select **Dot Plot** or **Histogram** and move the cursor onto the blank worksheet

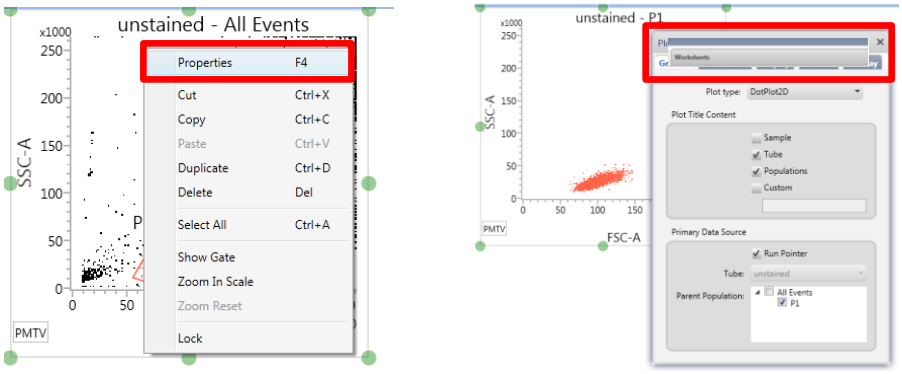




- to duplicate the plot, right mouse click on the plot

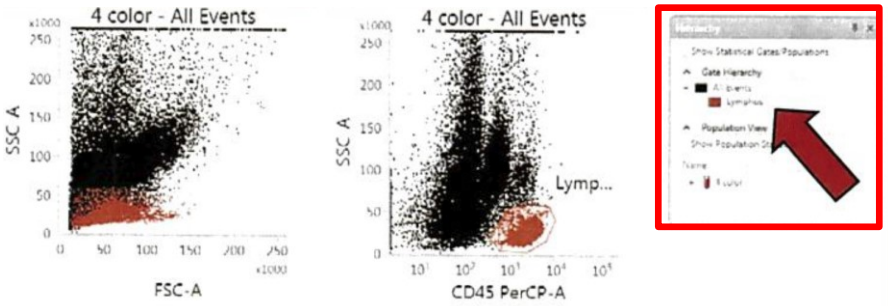


- to change plot properties, select the plot, right mouse click **Properties** (e.g. change the parent population)

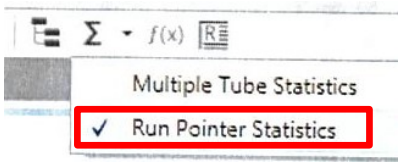


4.5 Display Hierarchy and statistic

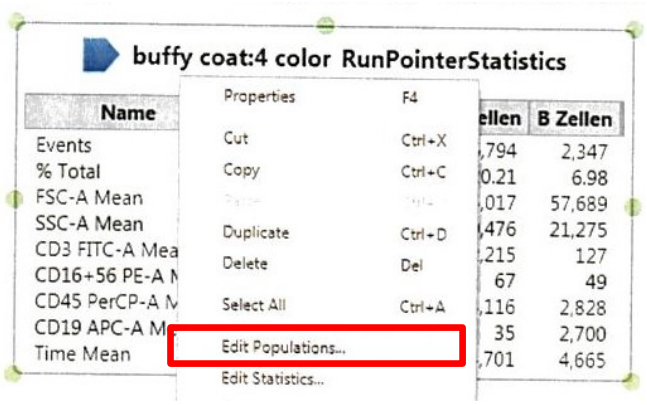
- shows the population hierarchy



- to create statistics select the symbol in the menu list

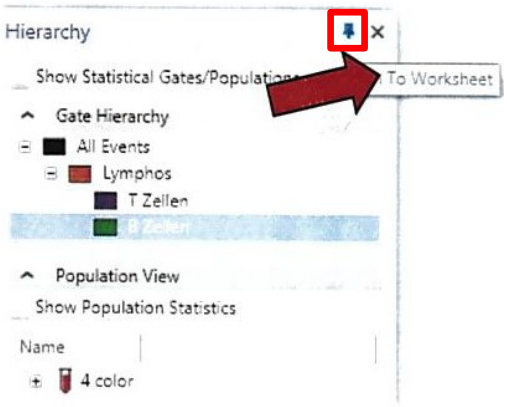


- **Multiple Tube Statistics:** summarize values from several tubes and
- **Run Pointer Statistics:** display values related to the currently selected tube
- select **Run Pointer Statistics**



- to change the statistic view right click into the statistic window **Edit Population** or **Edit Statistic**

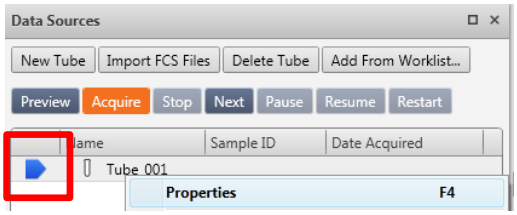
- to fix the hierarchy window on the worksheet use **Pin to Worksheet**



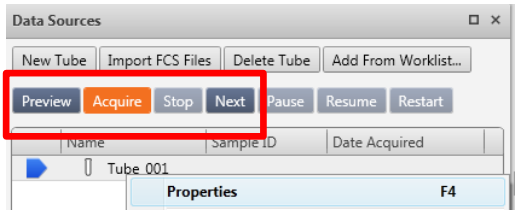
5. Optimizing FSC, SSC, Threshold and PMT Voltages

5.1 Optimizing FSC, SSC and Threshold

- verify that all needed plots are visible
- check if your tube is activated

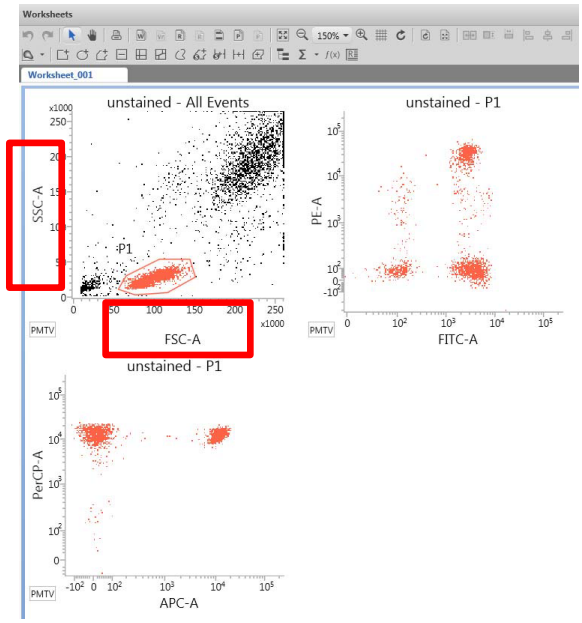


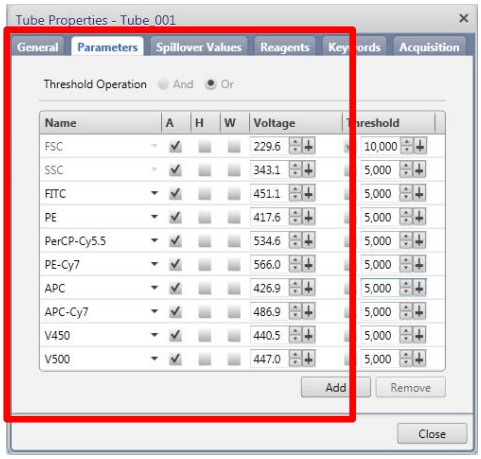
- remove the water tube from the SIP
- gently tab the tube to mix your **negative tube** and put your sample tube on the SIP (Sample Injection Port)
- press **Preview** to start the measurement (do not click Acquire!!!!)
- set up FSC, SSC and Threshold if necessary
- **Stop** the measurement



- replace the tube from the SIP
- wait until the automatic SIT-Flush is done
- load a tube with positive control to the SIP
- press **Preview**

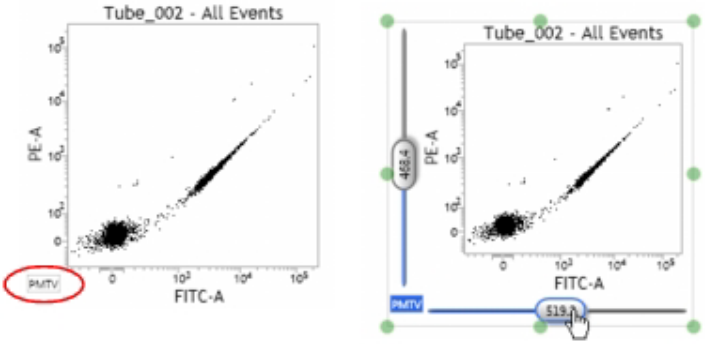
- check if FSC, SSC and Threshold fit for the positive control
- unload the sample and place the water tube on the SIP





5.2 Optimizing PMT Voltages for Fluorescence Parameter

- verify that all populations are on scale for each parameter in the corresponding plots
- press **Preview** to start the measurement
- adjust the PMT voltages if necessary
  NOTE: The compensation is automatically recalculated when you adjust PMT voltages
- adjust PMT voltages in a plot: click the PMTV button in the lower-left corner of the plot to enable the sliders



- drag the slider control for each axis parameter in the plot
- the PMTV value is displayed on the slider control
- click the PMTV button again to hide the slider control

- **Stop** the measurement
- remove the negative sample tube from the SIP
- wait that the SIT-flush is complete
- load the positive control sample tube and click **Preview**
- verify that all the populations are on scale for each parameter and adjust the PMT voltages if necessary
- press **Stop**
- remove the tube from the SIP and replace a tube with DI water

Important!!!!

The FACS Suite software has predefined tube settings for Lyse/Wash and No Lyse/No Wash as well as compensation values for both. This allows the samples to be measured accurately and reproducibly. Therefore -it is also usable for clinical applications.

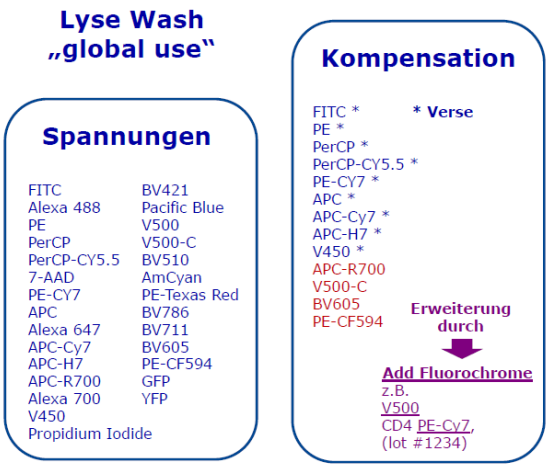
- Measure with predefined Lyse/Wash Settings: If you measure the experiment without **any** changes of the settings and parameters (tube settings from the system will be used) go on with the protocol

- Measure with your own tube settings: If changes to the Lyse/Wash instrument settings are necessary (e.g. PMT voltages, threshold, number of SIT-flushes) new tubes settings must be created (**Create New Tube Settings**).
  The compensation settings are still taken from the Lyse/Wash file and will be automatically recalculated.
  check if tube settings are changed --- **Tube Properties** --- is a **Star** behind the **Tube Setting** they are changed
  Go on with Page ……

- measure with your own compensation: If one has to add additional fluorochomes to the existing Lyse/Wash settings or uses tandem dyes (e.g. Pe-Cy7), own compensation settings has to be created, thus extending and partly overwriting the compensation values of the Lyse/Wash settings. One can still use compensation values of the Lyse/Wash Settings for standard fluorochromes (e.g. FITC, PE, PerCP). Add flurochromes to extend the Lyse/Wash Settings and create **Reference Settings** to create compensation values for these parameters.
  go on with Page …….

  Creating Reference Setting automatically creates corresponding Tube Settings!!!

Instrument Settings

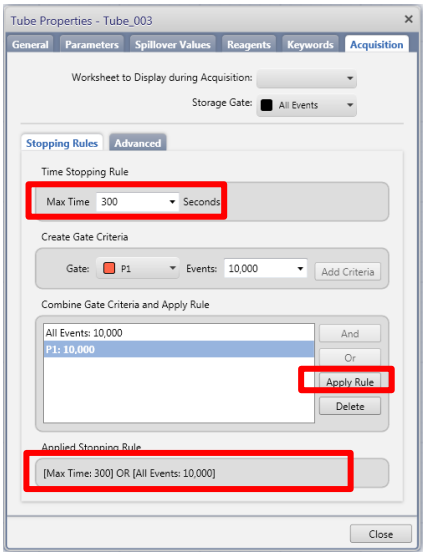


Picture shows all fluorochromes that are covered by the predefined PMT voltages within the Lyse/ Wash Settings (Left panel). If further flurochromes are added, reference settings must be created.
Right panel shows fluorochromes that are covered by the predefined compensations values within the Lyse/ Wash Settings. For all additional dyes the compensation has to be recalculated.

6. Acquire Data (Manual Port)

6.1 Setting Acquisition Criteria

- right click on the **Tube_001** to open the **Tube Properties**
- **Tube Name:** Rename the tube



Tube Properties:
Max Time: 300
Gate: P
Events:10.000
Combine Gate

- **Acquisition:** create a stopping rule (e.g. 10.000 P1)
- **Max Time**: enter 300
- **Create Gate Criteria:** select the Population P1 or P2,…
- **Add Criteria:** A new rule is added to the list of gate criteria
- select e.g. **P1:10,000** and click **Apply Rule**
- verify that the **Applied Stopping Rule** at the bottom of the window is [Max Time:300] OR [P1:10,000]
- close **Tube Properties** dialog
- create additional tubes by clicking **Next** in the **Data Sources** panel and rename the new tubes

*NOTE: This is a copy of the first tube including all tube properties. If you click **New**, this is a default tube*

6.2 Acquiring Data Manually

- verify that the run pointer is set to **Tube_001**
- load the negative tube on the SIP
- press **Preview**
- wait until event rate stabilize then click **Acquire** in the **Data Sources** panel
- monitor the acquisition in the **Acquisition Status** panel
- acquisition continues until one of the stopping rules are reached
- when acquisition is complete, the tube icon changes from black to green
- remove the sample tube from the SIP
- system performs a SIT-flush (loading port LED will blink **amber** when SIT-flush is in progress, then turn **green** when ready to load next tube)
- after SIT-flush, load the positive sample tube
- click **Next** in the **Data Sources** panel to move the pointer to the second tube
- click **Preview**, then **Acquire**
- remove the tube when acquisition is complete, and load a tube of DI water

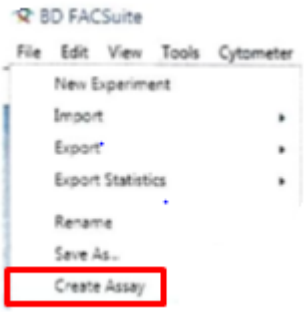
7. Save your Experiment

7.1 Save your Experiment

- Go to manage Experiments --- File --- Export Experiment --- With Data

7.2 Save your Experiment as an Assay

- **Menue List --- File --- Create Assay**

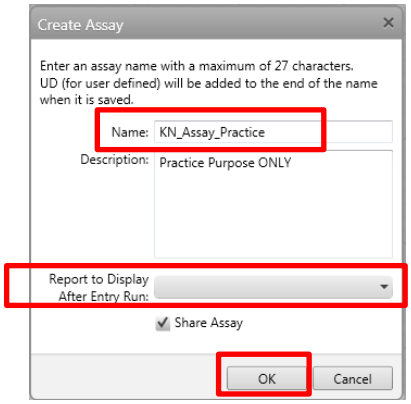


8. Creating an Assay from an Experiment

An Assay is a template that contains a set of tubes that share the same tube settings, worksheets and reports. Assays are run by a worklist using the universal loader. Once you´ve created an experiment, you can save it as an assay for reuse.

8.1 Open an existing experiment or create a new one

- select **File --- Create Assay**

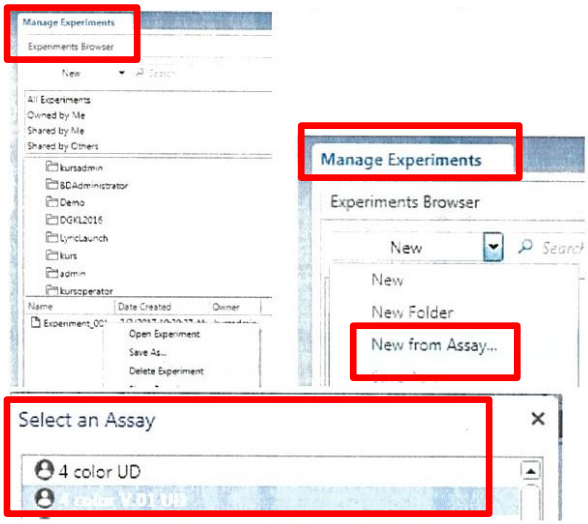


- enter a name
- select a report if needed
- click **OK**

Additional Information

8.2 Creating an Experiment from Assay

- select **File --- New from Assay**
  modify the experiment as needed
- select **File --- Create Assay**
  save the experiment as an assay and acquire data in a worklist
  alternatively, you can continue with data acquisition and analysis in the experiment workspace



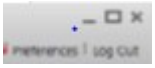
- saved assays are stored in the library
  Library --- Assays --- User- Defined --- Experiment name --- click **Ok**

9. Acquire Data in a Worklist

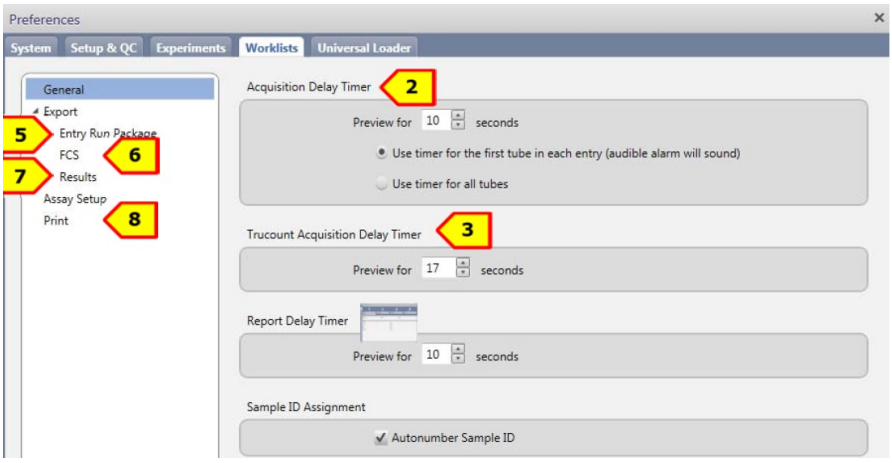
You can acquire data in a worklist using saved assays

9.1 Edit Worklist Preferences

- select **Tool --- Preferences** (on the upper right corner next to **Log Out**)



- select **Worklists**
- select **General** on the left side
- change **Acquisition Delay Timer** so that all tubes will preview for e.g. 20 sec before acquiring
- check or clear the auto number sample ID option

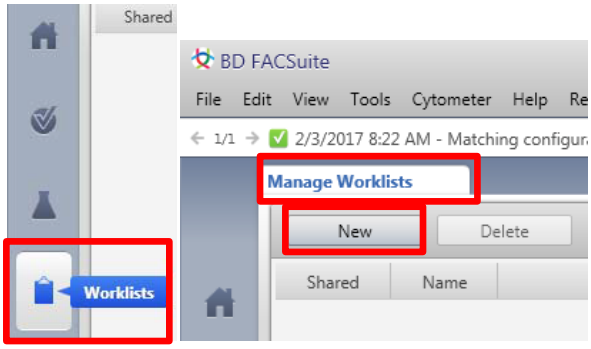


2. Acquisition Delay Timer: time until data gets saved, during this time you can change the threshold and move populations

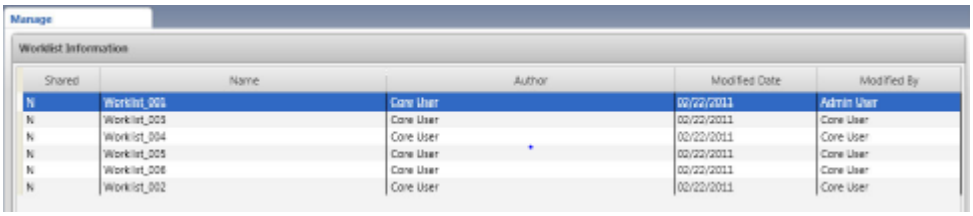
3. preview time for true count tubes
5. set up export criteria
6. FCS files
7. results, statistic files
8. print options

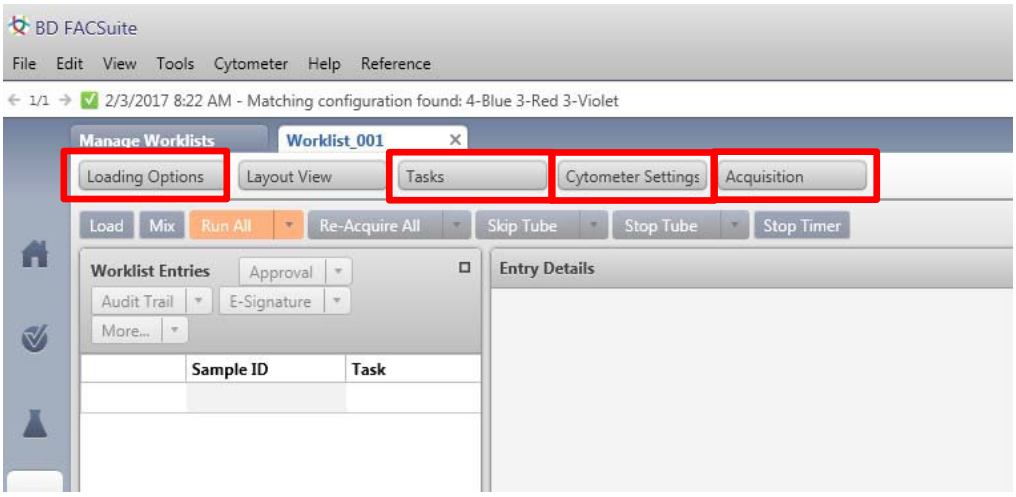
9.2 Creating a worklist

- click **Worklist** in the navigation bar



- **Manage Worklist** and click **New** (a blank worklist opens in a new tab)

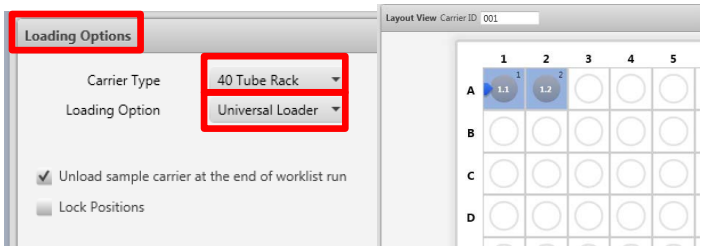




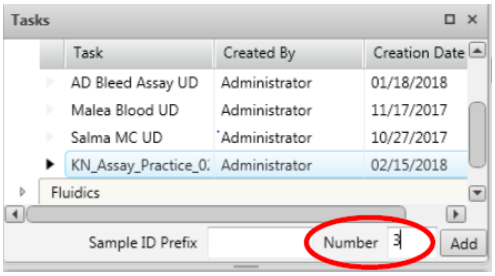
Loading options: define which carrier is used for the universal loader
Layout View: shows the position and the sequence on the carrier
Task:
Cytometer Settings: shows you the device settings
Acquisition: displays the process of the measurement
Worklist Entries: lists the tasks
Entry Details: shows worksheets of the assay

- select the loading options
    - **Loading Options:** select **Universal Loader**

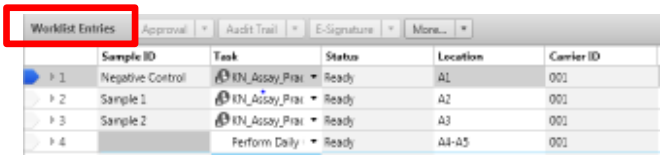
- select the carrier type that you would like to use: 40 tube rack, 30 tube rack, 96 Well Plate Standard round / flat bottom PS, …

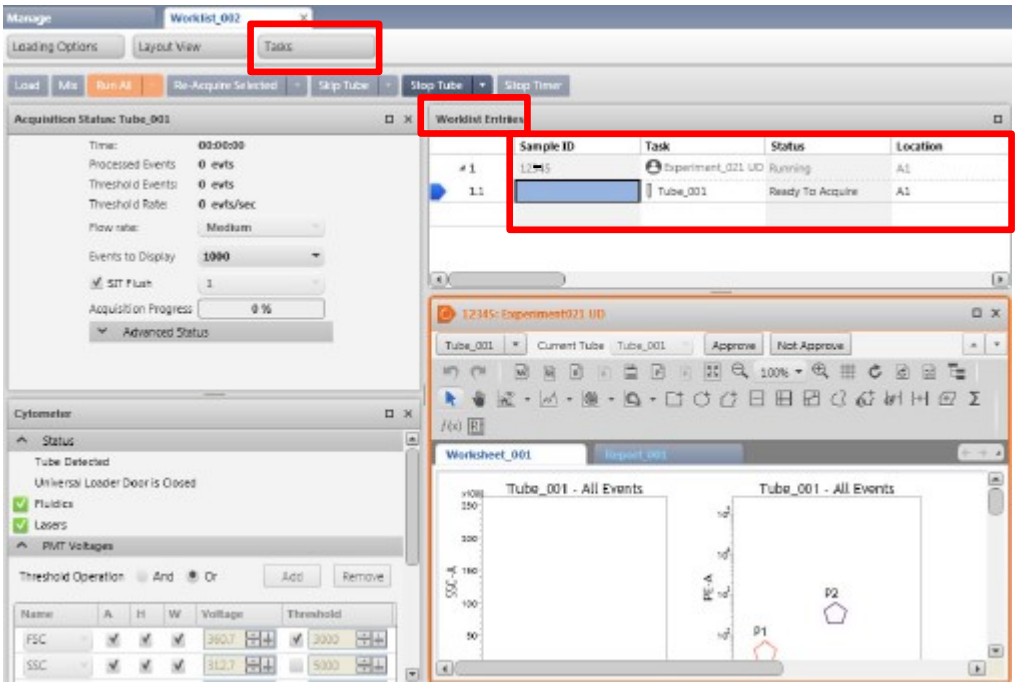


- use the **Task Panel** to select one or more assays to add to your worklist



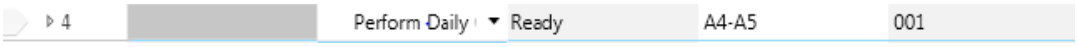
- enter information in the **Worklist Entries** panel





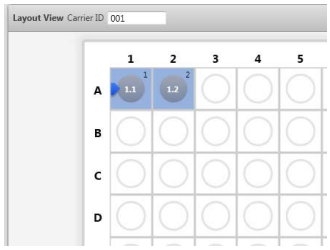
  - select your assay from **Task Menu**
  - enter your **Sample ID**
  - *use the same flow rate for all assays*

- at the end of your worklist set **Perform Daily Cleaning** **this works only if you measure your tubes in a Tube Rack but not with 96 well Plate measuring!!!!!!**

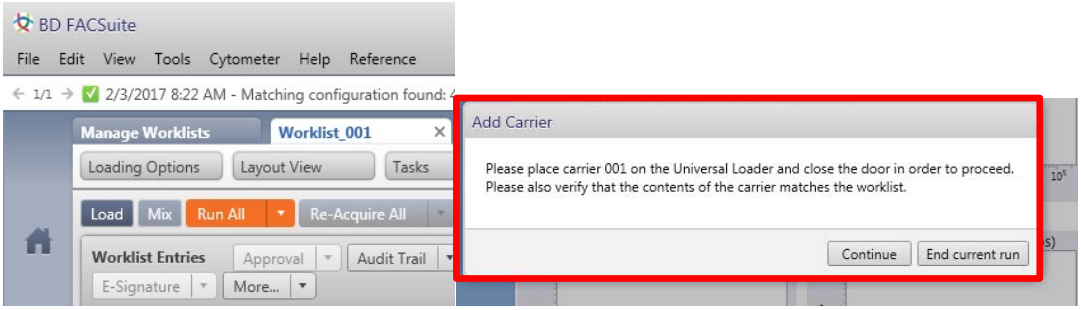


9.3 Acquiring Data in a Worklist

- load the carrier
  - samples should be placed in the same position as shown in the Layout View panel
  - *Mix your samples before you place them to the carrier*



- click **Load**
- place the carrier into the loader
- click **Continue**
- verify that a tube of DI Water is placed at the manual SIP
- click **Run All**
  - if already acquired data exist choose **Run from pointer** or **Run Selected**



- verify that all gates and PMT voltages are correct
  - click **Stop Timer** (the countdown timer stops, you have more time to preview the data)

- if necessary adjust voltages and gates
  - if you made some adjustments of the PMT voltages, you will be prompted to select how to apply those adjustments
- click **Resume**
- after the first worklist entry is complete (Tube 1), click **Stop Timer**
  - report delay is activated

    - *notice that you are now making changes to the gates in the analysis report*
- adjust the gates if necessary, then click **Resume**
- repeat the steps in the beginning for the remaining worklist entries
- after all samples have been acquired, remove the carrier and click **Continue**
-

9.4 Exporting a Worklist

- if necessary, close the worklist that you would like to export
- select the **Manage Worklists** tab
- select the worklist that you would like to export, then select **File --- Export Worklist --- With Data**
- navigate to the location where you would like to save the worklist, then click **Save**
- default location for the worklist export is *C:\BD Export\Assay Worklists*

10. Cleaning and daily Shutdown

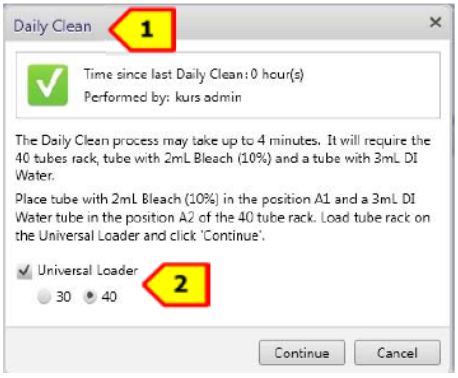
- prepare two tubes: one tube with 2 ml of clean solution and one tube with 2 ml DI water
- select **Cytometer --- Daily Clean**
- the cleaning can be done with the manual SIP or automatic via the universal loader

10.1 Manual cleaning with the SIP

- load the clean solution tube on the SIP
- when prompted, load the DI water tube
- click **Continue**
- dialog closes when the process is completed

10.2 Automatic clean with the Universal loader

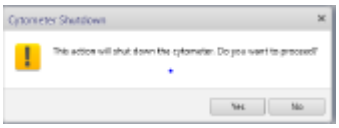
- choose **Cytometer --- Daily Clean**, select the universal loader and the 40 tube Rack



- place the tube with FACS Clean in position A1 and the DI water in position A2 of the 30 tube rack
- load the tube rack, if prompted click **continue**
- dialog closes if the process is done

10.3 Shut down

- leave a tube containing 2 ml DI water at the SIP
- select **Cytometer --- Shut Down**: cytometer will shut down



- exit out of BD FACS Suite software
- log out of PPMS and / or shut down the computer

Setup 1: Measure with Lyse/Wash settings

If no changes in the existing settings (Lyse/Wash settings) are made during the measurement, i.e. no changes to FSC, SSC, Voltages, Threshold, the experiment can be created, edited, saved as an assay and processed in the worklist as described above.

- Start the Daily clean
- Run the Daily QC
- You can start directly to choose your assay which you created the last time and start to create a new worklist or start with a new experiment

    - Create Experiment and Rename it
    - Select the tube properties and name your tube, and select the correct parameters in the parameter list
    - Check your negative sample if all parameters are lie within the scale
    - Create your Plots and Gaiting
    - Go to tube properties to set up your Acquisitions criteria
    - Go further on with the protocol…….

Setup 2 Measure with own created settings

If changes are necessary in the Lysis/wash settings for the next measurement (2nd measurement), e.g. by changing the voltage, FSC, SSC and threshold, the tube settings must be created

If you work the fist time with Assay/Tube Settings

1. Start the daily cleaning
2. Perform the QC (check if the right Bead Lot is set)
3. Create your experiment; rename it….
4. Go to tube properties; rename your tube, place your Parameters, Reagents inside
5. Place your negative sample under the SIP and go to Preview
6. Adjust your Parameter
7. Unload your sample from the SIP
8. Got to your tube--- press tube properties--- Gerneral and check your tube settings --- if a blue star is behind the tube settings the Lyse/wash settings got chanced for this case run the Assay and Tube Settings
9. The tube must not contain any saved data otherwise the option create tube settings cannot be selected. the tube can then be duplicated and all changes are copied
10. Go to tube right mouse click--- Create Tube Settings

11. Verify that you place the right CS&T Bead lot number under the SIP
12. Press Acquire
13. Enter the name of the tube settings
14. Now the blue star behind the tube settings in the tube properties is gone
    You find your new Settings in the Library--- Tube Settings--- User Defined
15. To adjust the settings for the other tubes in your experiment go to the next tube--- right click on properties--- General--- Select and select your saved tube settings and close the control panel
    - Go further on with the next tubes and repeat the step 15.
16. Measure you sample??????????????????
17. Save your Experiment as Assay--- File--- Create Assay
18. Save your Experiment--- File--- Export Experiment--- With Data

Important!

If the assay has been re-created, it will be current for 24 hours. For use on later days, an assay/tube settings setup must first be performed SEE....

19. Measure you samples in a Worklist

If you measure your samples the next time with your created experiment go further on!!!

Start the Assay and Tube Settings

- Choose Setup and QC
- Verify that the right CS&T Beat lot is use
- Choose in **Setup & QC Options** --- **Task: Assay/ Tube Settings Setup**
- Choose in **Assays / Tube Settings: Select**
- The Select Tube Settings and Assay Setup Reports window displays all existing tube settings in the respective assays
- **Select Run Setup** from the **Tube Setting** you want to use. You can also select **multiple tube settings** via **run setup** or go to **Run all Setup** then all existing Tube Settings will be adjusted
- **Click start**
- Load the tube with the CS&T Beads under the manual SIP
- Click Continue
- The screen shows you Assay/Tube Setting Setup successfully completed Would you like to see the report Yes/No Yes
- Assay/Tube Settings Setup is successfully completed