

## Lyric

## **Operation Protokoll BD FACS Lyric**

## **FCF-Berg**

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Index	Page
Cytometer Overview	
1. Starting up the System	5
2. Daily Start up	5
3. Daily Performance Quality Control (QC)	5
<ul> <li>4. Experiment Setup</li> <li>4.1 Create a New Experiment</li> <li>4.2 Delete or Add Parameters</li> <li>4.3 Acquisition Window</li> <li>4.4 Plot creation and Gating</li> <li>4.5 Display Hierarchy and Statistic</li> </ul>	6 7 8 9 10
5. Optimizing FFC, SSC, Threshold and PMT Voltages 5.1 Optimizing FSC, SSC and Threshold 5.2 Optimizing PMT Voltages for Fluorescence Parameter	11
6. Acquire Data 6.1 Setting Acquisition Criteria 6.2 Acquire Data manually	
7. Save Experiment 7.1 Save your Experiment 7.2 Save your Experiment as an Assay	
8.0 Creating an Assay from an Experiment 8.1 Open an existing experiment or create a new one 8.2 Creating an Experiment from Assay	
<ul> <li>9. Acquire Data in a Worklist</li> <li>9.1 Edit Worklist Preferences</li> <li>9.2 Create a Worklist</li> <li>9.3 Acquiring Data in a Worklist</li> <li>9.4 Exporting a Worklist</li> </ul>	
Setup1; Measure with Lyse/Wash Settings Without changing any Settings Setup2; Measure with own created Settings	

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<sup>™</sup> 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<sup>™</sup> 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

./	Performance QC for Normal Fluidics Mode successfully completed.
<u>v</u> _	Performance QC for High Sensitivity Fluidics Mode successfully complete
	Assay / Tube Settings Setup successfully completed.
	Would you like to view the Performance QC report(s)?

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 1<sup>st</sup> 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

	Manage Experiments
	Experiments Browser
	New 💌 🔎
Π	All Experiments Owned by Me
51	Shared by Me
	Shared by Others
-	BDAdministrator
X	DemoUser
	BDJH01
	kursadmin

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

😾 BD FACSuite	
File Edit View Tools Cytometer H	Rename Experiment X
New Experiment	Enter an experiment name with a maximum of 60 characters.
Export +	Experiment Name: Fall 1, LyseWash Settings, 4 color
Export Statistics	
Rename	
Save As	

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

Acquisition Status     Image 00:00:00       Processed Events 0 evits     Processed Events 0 evits       Threshold Events 0 evits     Image 00:00:00       Threshold Events 0 evits     Image 00:00:00       Bow Rate     Medium       Events to Display     1000       Number of SIT Flukes 1     200       Acquisition Progress     0 %       V     Advanced Status	<ul> <li>Ξ: Q. 100% - Q. #</li> <li>Ξ: Σ - f(x) R#</li> <li>rents</li> </ul>
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Proceeds Genets 0 evits Threshold Foreits 0 evits Threshold Fate 0 evit/sec Foreits to Digitaly 1000 • Number of SIT Fluthes 1 • Acquisition Progress 0% * Advanced Status Data Sources • ×	rents
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Tube_001 - All	vents
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Vertilities 1 1000     Vertilities 1     Acquisition Progress 0 %     Acquisition Progress 0 %     Advanced Status     Jacquisition Progress 0 %     Jacquisition Progress 0 %	
Number of SI Plushes     *       Acquisition Progress     0 %       * Advanced Status     4       Data Sources     0 ×	
Acquisition Progress 0 %	
Advanced Status	
Data Sources	
Data Sources D X	
Data Sources	۰
New Tube Import FCS Files Delete Tube Add From Worklist	
Preview Acquire Stop Next Pause Resume Restart	
0 50 100 11	200 250

#### 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

Data Sources		_
New Tube Import FCS Files Delete Tube Add From Worklist	Tube Properties - Tube_001	×
Preview Acquire Stop Next Pause Resume Restart	General Parameters Spillover Values Reagents Keywords Acquisitio Tube Name: Unstained	n
Name Sample ID Date Acquired		
Properties F4	Tube Settings:         Lyse Wash         Select           Total Events:	
	Acquisition Date: Cytometer Name:	
	Close	

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

Threshold Operati	on	An	d 🖲	Or			
Name		A	Н	w	Voltage	Threshol	d
FSC		~		111	229.6	✓ 10,000	++
SSC		~		10	343.1 🛟 🕂	5,000	÷+
FITC	*	1	10	10	451.1 ++	5,000	÷+
PE	•	1		10	417.6	5,000	÷4
PerCP-Cy5.5		~		10	534.6	5,000	÷+
PE-Cy7	•	~		10	566.0 ++	5,000	÷4
APC	-	~		10	426.9 🛟 🕇	5,000	÷+
APC-Cy7	*	~		11	486.9 井	5,000	++
V450	*	~	-	10	440.5	5,000	÷4
V500	-	~		10	447.0 🛟 🕇	5,000	÷+

Threshold Op	eration	An	d 🖲	Or					
Name		A	Н	W	Voltag	je	Th	reshold	ł
FSC	×	~	100	100	229.6	÷[+]	~	10,000	÷+
SSC		~		111	343.1	++	100	5,000	÷4
FITC	•	~	10	100	451.1	÷+		5,000	÷4
PE	•	1	100	10	417.6	÷[+		5,000	÷+
PerCP	•	~	10	10	534.6	÷[+		5,000	÷[+
APC	•	~	10	10	426.9	÷[+		5,000	÷+
							Add	R	emove

**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

eneral Parameters	Spillover Values Reagents	Keywords Acquisition
Tube Name:	Tube_001	
Tube ID:		
Sample ID:		
Tube Settings:	Lyse Wash	Select
Total Events:		
Acquisition Date:		
Cytometer Name:		

#### 4.4 Plot creation and Gating

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

Worksheets								
<b>1</b> 7 (21	WR	P	КЛ ( КЛ	۹.	100%	• (	Ð,	##
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/		$\mathbf{i}$			•			
Dot Plot	Histogram	Con	tour Plo	ot	Der	nsity	Plo	t



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

Worksheets				
	R P KA	⊖ 100% - ⊕	###	
💽 🖷 🗠 🗠	<u>- @</u> - <u></u> -	t d d E	E	
			$\langle \rangle$	
Rectancgle gate	Ellipse gate	Poligon gate	Interval gate	Quadrant gate

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



#### 4.5 Display Hierarchy and statistic



• 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
- buffy coat:4 color RunPointerStatistics Properties F4 Name ellen B Zellen Cut Ctrl+X Events ,794 2,347 % Total Copy Ctrl+C 0.21 6.98 FSC-A Mean ,017 57,689 SSC-A Mean 476 21,275 Duplicate Ctrl+D CD3 FITC-A Mea 215 127 Delete Del CD16+56 PE-A N 67 49 CD45 PerCP-A N Select All Ctrl+A 116 2,828 CD19 APC-A M 35 2,700 Edit Populations... Time Mean 701 4,665 Edit Statistics...
- 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

Hierarchy	<b>₽</b> ×
Show Statistical Gates/Popula	To Worksheet
<ul> <li>Gate Hierarchy</li> </ul>	
😑 🛄 All Events	
😑 🧱 Lymphos	
T Zellen	
B Zeler	
<ul> <li>Population View</li> </ul>	and a set of the
Show Population Statistics	
Name	
🗄 🧃 4 color	

#### 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

Data Sources		□ ×					
New Tube Import FCS File	s Delete Tube	Add From Worklist					
Preview Acquire Stop	Next Pause	Resume Restart					
Name	Sample ID	Date Acquired					
Tube 001							
Prope	erties	F4					

- 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



Threshold Opera	ation 🔵 A	nd 🖲	) Or		
Name	A	Н	w	Voltage	Inreshold
FSC			111	229.6 ++	10,000 + +
SSC			10	343.1 ++	5,000 ++
FITC		1	10	451.1 井	5,000 ++
PE			10	417.6	5,000 ++
PerCP-Cy5.5			10	534.6	5,000 ++
PE-Cy7	- /		10	566.0 ++	5,000 ++
APC	- /		10	426.9 +	5,000 ++
APC-Cy7				486.9 井	5,000 ++
V450			10	440.5	5,000 斗
V500		1	10	447.0	5,000 🗘 🕂
				Δ	d Remove

#### 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

ral Parameters Spillover Values Reagents Keywords Acquisition Worksheet to Display during Acquisition: Storage Gate: All Events topping Rules Max Time 300 Seconds Create Gate Criteria Gate: P1 Events: 10,000 Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 Add Criteria Combine Gate Criteria and Apply Rule Delete Angliert Stongings Rule (Max Time: 300) OR (All Events: 10,000 Combine Gate (Max Time: 300) OR (All Events: Close	ral Parameters Spillover Values Reagents Keywords Acquisition Worksheet to Display during Acquisition: Storage Gate: All Events  opping Rule Max Time 300 Seconds Create Gate Criteria Gate: P1 Events 10,000 Add Criteria Combine Gate Criteria and Apply Rule All Events 10,000 And Or Apply Rule Delete
Worksheet to Display during Acquisition:	Worksheet to Display during Acquisition:         Storage Gate:         All Events         opping Rules:         Max Time:         300         Seconds         Create Gate Criteria         Gate:       P1         Events:       10,000         Add Criteria         Combine Gate Criteria and Apply Rule         All Events:       0,000         P1:       10,000         And         Or       Apply Rule         Delete:       Delete:
Storage Gate: Alt bents    topping Rules  Advanced  Time Stopping Rule  Max Time 300   Seconds  Create Gate Criteria  Gate: P1  Events: 10,000  Add Criteria  Combine Gate Criteria and Apply Rule  Add Criteria and Apply Rule  Add Criteria and Apply Rule  Delete  Annied Sthonnion Rule  [Max Time: 300] OR [All Events: 10,000]  Close	Storage Gate: All Events    apping Rules  Advanced  Time Stopping Rule  Max Time 300  Seconds  Create Gate Criteria  Gate: P1  Events: 10.000  Add Criteria  Combine Gate Criteria and Apply Rule  All Events: 10.000  And Or  Apply Rule  Delete  Delete
topping Rules Advanced Time Stopping Rule Max Time 300 • Seconds Create Gate Criteria Gate: P1 • Events: 10,000 • Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 • And P1 = 10,000 • Or Apply Rule Delete Annied Stopping Rule [Max Time: 300] OR [All Events: 10,000] Close	opping Rules Advanced Time Stopping Rule Max Time 300 • Seconds Create Gate Criteria Gate P1 • Events 10,000 • Add Criteria Combine Gate Criteria and Apply Rule All Events 10,000 And Or Apply Rule Delete
Time Stopping Rule          Max Time 300 • Seconds         Create Gate Criteria         Gate:       P1 • Events: 10.000 • Add Criteria         Combine Gate Criteria and Apply Rule         All Events: 10.000       And         P1:       Events: 10.000         And         P1:       Events: 10.000         And         P1:       Events: 10.000         And         P1:       Events: 10.000         And         P1:       Events: 10.000         Core       Apply Rule         Delete       Annied Stopping Rule         (Max Time: 300] OR (All Events: 10.000)       Close	Time Stopping Rule       Max Time     300     Seconds       Create Gate Criteria     Gate:     P1       Combine Gate Criteria and Apply Rule       All Events:     10,000       P1:     10,000       And       Orr       Apply Rule       Delete
Max Time 300 Seconds Create Gate Criteria Gate P1 Events: 10.000 Add Criteria Combine Gate Criteria and Apply Rule All Events: 10.000 And P1: 10.000 Or Apply Rule Delete (Max Time: 300] OR (All Events: 10.000) Close	Max Time 300 • Seconds Create Gate Criteria Gate P1 • Events: 10,000 • Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 And P1: 10,000 Or Apply Rule Delete
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Gate: P1 Events: 10,000 Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 And P1: 10,000 Or Apply Rule Delete (Max Time: 300] OR (All Events: 10,000) Close	Gate: P1 • Events: 10,000 • Add Criteria Combine Gate Criteria and Apply Rule All Events: 10,000 And P1: 10,000 Or Apply Rule Delete
Combine Gate Criteria and Apply Rule          And         P1: 10.000         P1: 10.000         And         Or         Apply Rule         Delete         Annied Stromics Rule         [Max Time: 300] OR [All Events: 10.000]	Combine Gate Criteria and Apply Rule All Events: 10,000 P1: 10,000 Or Apply Rule Delete
All Events: 10.000 P1: 10.000 Or And Or Apply:Rule Delete Anniled Stransion Rule [Max Time: 300] OR (All Events: 10.000] Close	All Events: 10,000 And P1: 10,000 Cr Apply Rule Delete
Cor Apply Rule Delete (Max Time: 300] OR (All Events: 10,000) Close	P1: 10,000 Or Apply Rule 1 Delete
Apply Rule Delete (Max Time: 300) OR (All Events: 10,000) Close	Apply Rule Delete
Analied Stanning Bule [Max Time: 300] OR [All Events: 10,000] Close	Delete
Annlied Stranning Rule [Max Time: 300] OR [All Events: 10,000] Close	
[Max time: stulj Uk (All Events: 10,000]	Applied Stopping Rule
Close	[Max Time: 300] UK (All Events: 10,000]
Close	Close
	Close
	Max Time: 200
Max Time: 200	
Max Time: 300	Gate: P
Max Time: 300 Gate: P	Events:10.000
Max Time: 300 Gate: P Events:10.000	

- 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

File	Edit	View	Tools	Cytometer
	New E	perim	ent	
	Impor	t .		
	Export	e		
	Export	: Statisti	ics	
	Renan	ne	•	
	Seve A	ls		
	Create	Assay	٦.	

#### 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



#### **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

All Experiments	
Gwned by Me Shared by Me	
Participation and a second sec	Manage Experiments
E BDAdministrator	
Pi Demo	Experiments Browser
E DGKL2016	
Elynclaunch Cent	New 💌 🗩 Sea
Prikursoperator	New
Name Date Created Owner	New Folder
Den Experiment	
Save As	New from Assay
Delete Experiment	M
elect an Assav	×

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

Preferences		×
System Setup & QC Experiments	Worklists Universal Loader	
General Export 5 Entry Run Package FCS 6 Results Assay Setup	Acquisition Delay Timer 2 Preview for 10 * seconds Use timer for the first tube in each entry (audible alarm will sound) Use timer for all tubes	
Print 8	Trucount Acquisition Delay Timer	
	Report Delay Timer Preview for 10 * seconds	
	Sample ID Assignment	

**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)

Fordist Information									
Shared	Name	Author	Modified Date	Modified By					
N	Workint_005	Care User	02/22/2011	Admin Uter					
N	Worklint_005	Core User	02/22/2011	Core User					
N	Worklist_004	Core User	02/22/2011	Core User					
N	Worklint_005	Core User	02/22/2011	Core User					
N	Workint_006	Core User	02/22/2011	Core User					
N	Worklist_002	Core User	02/22/2011	Core User					

#### BD FACSuite File Edit View Tools Cytometer Help Reference ← 1/1 → 🗹 2/3/2017 8:22 AM - Matching configuration found: 4-Blue 3-Red 3-Violet Manage Worklists Worklist 001 × Loading Options Tasks Cytometer Settings Layout View Acquisition Re-Acquire All Skip Tube Stop Tube Load Mix Stop Timer A Worklist Entries Approval \* **Entry Details** Audit Trail \* E-Signature \* More... \* 3 Sample ID Task

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

Tas	Tasks 🗆 ×								
		Task	Created By	Creation Date 🛋					
		AD Bleed Assay UD	Administrator	01/18/2018					
		Malea Blood UD	Administrator	11/17/2017					
		Salma MC UD	Administrator	10/27/2017					
	۲	KN_Assay_Practice_0	Administrator	02/15/2018					
⊳	Fluidics								
•									
		Sample ID Prefix	Numb	er 3 Add					

• enter information in the Worklist Entries panel

Worldist I	Entries	Approval	* Audit Trail *	E-Signature   *	Mone *	
	Samp	fe ID	Task	Status	Location	Carrier ID
▶ 1	Negat	ive Control	O KN_Assay_Proc •	Ready	AL	001
) F.2	Sample	el.	PIN_Assay_Prac •	Ready	AZ	001
1.13	Sample	e 2	RN_Assay_Prac •	Ready	A3	001
1.14			Perform Daily	Ready	A4-A5	001



- o select your assay from Task Menu
- o enter your Sample ID
- o 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!!!!!

	> ▶ 4	Perform Daily 🔻	Ready	A4-A5	001
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#### 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)

Load Mix Run All 🔻 Re-Acquire All 🔻	Skip Tube 🔹	Stop Tube 🔹	0:07 Stop Timer
-------------------------------------	-------------	-------------	-----------------

- 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** 
  - $\circ$  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
   Cronerer Stations



- 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.
- 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