# LSRFortessa Operation Protocol

#### Make sure the following actions have been taken before running your samples.

- Make sure the waste tank is empty and refilled with 500 ml clean (see page 16 for details)
- ✤ Make sure the FACS Flow container is fully filled (see page 16 for details)
- 1. turn on the computer
  - ✤ Login into Windows using User Name Admin and password: BDIS#1.
- 2. Turn on the FACSflow supply system
- 3. Turn on the main power switch of the cytometer (Green button right side)
- ✤ Please make sure that the green lights on both instrument BD LSR-Fortessa and FACS Flow system are actually "ON".

Wait 30 minutes for the lasers to warm them up before run your samples

- Check Fluidics:
  - > Check if the BD LSR Fortessa fluidic system is functioning properly:
    - Remove the tube with MQ water from the sip (Sample Injection Port);
    - Push "RUN" and "High" fluidic control buttons;
    - Check if the buffer starts dripping from the sip:
      - If "yes" please proceed to the step IIb;
      - If "not" please push the "Standby" button immediately and report the problem to FCF Staff (see the notes on the end of the document).
  - Check if the trap filter attached to the pressurized plastic tank is free of air bubbles:
    - If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.
    - Note: When removing air bubbles, do not vigorously shake, bend, or rattle the sheath filter - you might damage it.
    - Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.
    - Tilt the filter and verify that no trapped air remains in the filter.
    - Repeat steps 3 and 4 until no air is observed in the filter.



#### > Check the sheath line for air bubbles.

- Open the roller clamp at the fluidics interconnect (if necessary) to bleed off any air in the line. Collect any excess fluid in a waste container.
- Close the roller clamp.

## 4. Launching the BD FACS DIVA Software

✤ Log in FACS DIVA software with your personal login name and password.

2 1	
NOI	
User Name: 😽	User Name
Deserved 1	*****

Always click "Use CST Settings" when pop-up message as below appears.

CST Mismate	eh	
The settin Do you w	ngs from CST are different ant to use the CST values show this message again f mber my decision.	from those on the cytometer. ? or current login session.
Details>>	Use CST Settings	Keep Current Settings

- 5. Creating and working with experiments in BD FACS DIVA Software
- Browser toolbar 
  click New Experiment & rename the experiment if necessary. or import an old one and duplicate without data.



Select New Specimen expands the Specimen to show Tube 001. Highlight the tube with the Tube Pointer.



Right click on Specimen\_001 > Rename if necessary; Right click on Tube\_001 > Rename if necessary.



Click the New Tube bottom to create new tubes. Rename them if necessary.



Select Cytometer Settings > Parameters > Delete unnecessary parameters on the Inspector Window.

Cytometer Settings	Parameters bresh	old Batio Comp	ensation				_
Global Sheet1	Parameter	Voltage	Log	A	н	W	
	· FSC	524		1			1
	• 55C	392		4			
	* FITC	562	~				
	* PE	536	~				
	* PerCP-Cy5-5	608	~				
	* PE-Cy7	804	~				
	· APC	648					
	# APC-CV7	507					
	Add	E			Delete		

FSC (measure Cell Size) and SSC (measure Cell Granularity) are a MUST for all kind of analysis and they should be kept in linear scale. Please check 'A'rea, 'H'eight and the 'W'idth of FSC and SSC. 'Log' and 'A'rea boxes should be checked for fluorescence channels except for cell cycle and/or DNA analysis.

Parameters Thresh	old Ratio Comp	ensation				_
Parameter	Voltage	Log	А	н	W	
· FSC	524					•
• SSC	392		V	V	V	
· FITC	562	~	Y			1
· PE	536	4				
· APC	648	~				

- Choose Experiment > Experiment Layout and define labels for each parameter.
  - Select the column of fluorescence channel and enter a label in the Quick Entry

	Labor	nora
Experiment	Populations	Worksheet Cy
New F	⁼older	Ctrl+N
New E	Experiment	Ctrl+E
餐 New :	5pecimen	Ctrl+M
New 1	ſube	Ctrl+T
🖨 New (	Eytometer Set	tings
Import Cy	tometer Setti	ngs
📔 New i	Global Worksh	eet
📑 New I	Plate	Ctrl+Y
Open Exp	eriment	Ctrl+0
Close Evr	eriment	CERTERN
Experime	nt Layout	
Compone	stion Solup	

I abel field

<b>Experi</b> r Labels <sub>Ke</sub>	nent Layout iywords Acquisition		
COuick F Label	CD3		
	Name	Label	Label
	🔚 Unstain	FITC CD3	PerCP-Cy5-5
•	🔓 FITC	FITC CD3	PerCP-Cy5-5
•	🍹 PE	FITC CD3	PerCP-Cy5-5
	🔚 PerCP	FITC	PerCP-Cy5-5

Select **Dot Plot** or **Histogram** move the cursor onto the **blank worksheet**.

🞬 Global Worksheet - Global Sheet1 👘	🞬 Global Worksheet - Global Sheet1
	🔛 💩 🔁 🗟 🖄 🙆 🖾 🗩 🗩 🗗 🗗 🕵 🤶
Global Sheet1 Dot Plot	Specimen_001-Tube_001
	Count Count
🔛 Global Worksheet - Global Sheet1	50 100 150 200 250 FSC-A (× 1.000)
Global Sheet1 Histogram	

✤ Right click on a plot > Duplicate to create another plot of the same type.

Global Sheet1			
Specim	en_001-Tube_001		
() & ·			
Z I			
- 8-	Show Population Hierarchy	Ctrl+G	
3	Create Statistics View	Ctrl+R	
못확	Show Populations	۲.	
280	Scale to Population	,	
=	Show Gate		
_	Bring to Front		
2	Send to Back		
-			
50	Dunicate	Chil+D	
115513, 218690)	Dacto .	Colum	
	r dolo	1000st-A	
	💥 Cut	Ctrl+X	
	Copy	Ctrl+C	
	Delete	Delata	

Select each individual axis, and choose from a list of offered parameters the preferred one.







- Right click on the plot > Show Population Hierarchy
  - Right click on the plot > Create Statistics View 
     Iright click on the statistics view
  - Edit Statistics View



Select Statistics tab > tick mean of FSC-A and FSC-H > tick the mean of the 'A'rea of the parameters > click OK



#### 6. Procedures for sample acquisition

- Press "RUN" and "LO" on fluid control panel.
- Gently tab the tube to mix your sample & put your sample tube on SIP (Sample injection port) & Run the unstained/negative control sample before other sample tubes.
- Do not run the machine without putting a tube filled with fluidics on SIP
- Acquisition Dashboard> Acquire Data
- NOTE: The sample is acquired the whole time being on the SIP!

Current Activity					
Active Tube/Well	Thres	hold Rate	Stopping Gate E	Events	Elapsed Tim
Tube_001	0 ev	tis	0 evt		00:00:00
Basic Controls					
📲 Next Tube	<b>A</b>	acquire Data	Record Data	•	lestart
Acquisition Setup					
Stopping Gate:	All Events	Events To Re	cord: 10000 ev	vt 🔽 Stopp	ing Time (sec):
Shavana Caba	All Events	Events To Dis	nlaw: 1000 ev	+ +	

- Identify the population of interest by adjusting the voltage of FSC and SSC on Parameters
- Press Restart to accelerate the changes.

		-000		I PARTY A		-
Parameter	- tokogo		A	н	W	
<ul> <li>FSC</li> </ul>	563		<b>~</b>		Image: A start of the start	_
SSC	461 🤶			<b>~</b>		
• FITC	627	-40				
• PE	615					_
PerCP-Cy5-5	746	-200				_
• PE-Cy7	730					
• APC	712	0				
APC-CY/	555					
Acquisition Dashboard	]	Ļ				
Acquisition Dashboard Current Activity	]					
Acquisition Dashboard Current Activity Active Tube/Well	Threshold Rate		Stopping	Gate Events		El
Acquisition Dashboard Current Activity Active Tube/Well Tube_001	Threshold Rate	Ļ	Stopping O evt	Gate Events		Eli
Current Activity Active Tube/Well Tube_001 Basic Controls	Threshold Rate		Stopping <b>0 evt</b>	Gate Events		EI
Acquisition Dashboard  Current Activity  Active Tube/Well  Tube_001  Basic Controls  I Next Tube	Threshold Rate 4 evt/s		Stopping O evt	Gate Events	Restart	EI:

- Cytometer > Laser & Adjust the FSC Area Scaling until the mean of FSC-A and FSC-H are <u>APPROXIMATELY THE SAME</u>.
- Return to Parameters tab and finely re-adjust voltage of FSC and SSC.

tatus Parameters Threshol	Laser Compensation Ratio	
Status	Measured	Reference
Blue Laser Current	1.26	1.28
Blue Laser Power	20.20	20.19
Red Laser Power	17.10	18.00
FSC Area Scaling: 0.50	<u> T</u>	
Name	Delay	Area Scaling
Name	Delay 0.00	Area Scaling 1.40

Adjust voltage of each of the fluorescent channels; preferably the peak of the "negative" population (mean in statistic view) of interest is greater than ZERO but less than 10<sup>2</sup>.

tat s Parameters Threshold Laser	Compensation	Ratio		-800
Parameter	Voltage	Log	A	
• FSC	526		1	-600
· SSC	395		1	4
• FITC	555	1	1	400
• PE	534	~	5	200
• APC	643		12	

- Click Stop Acquiring on Acquisition Dashboard and replace your sample with DI H<sub>2</sub>O.
- Repeat above steps with the positive control sample tubes. Adjust the voltage of corresponding channels if their signal peaks are **outside** the limit of the histograms.

## 7. Creating Gates

Set the current tube pointer to the following tube and use Polygon Gate to gate the targeted cells.



Gate the cells of interest according to the following sequence by using:



To define fluorescence positive signal, interval gate (P4, P5,...) beyond negative peak of fluorescent channels in histogram plot of unstained samples can be created. For over multiple fluorescence channels, quadrant gate could be created to define single/double positive signals (Q1; Q2; Q3; Q4....).



\* Note that *P1* is the children of *All Events* and the parent of *P2* population; *P2* population is the children of *P1* population and the parent of *P3* population and the grandparent of *P4*, *P5*, and *P6* populations. <u>Thus, on the hierarchy table, users should highlight the *P1* population when a gate for *P2* population is drawn, the *P2* population when a gate for *P3* population when a gate for *P4*, *P5*, and *P6* populations are drawn.</u>



### 8. Recording data for all samples

- Gently tab the tube to mix your sample then put your sample tube on SIP.
   Run the unstained sample before other sample tubes.
- Press "RUN" and "LOW" on fluid control panel.

#### Acquisition Dashboard > Acquire data > Record data

Active Tube/Well		shold Rate	Stopping Gate Events		Elapsed Tim	
Tube	001 0 e	vt/s	0 evt		00:00:00	
Basic Controls	_		1010010			
📲 Next Tub	. 8	Acquire Data	Record Data		Retat	
Acquisition Setup						
Stopping Gate:	Al Events	Events To Record:	10000 evt	~	Stopping Time (sec):	

- Please pay attention to the sample tube to make sure it will not run dry!
- Click "Stop Acquiring" to stop acquire the data if necessary then unload your sample.
- Repeat above steps for each sample.

#### 9. Machine Cleaning

#### Cleaning procedure between each user is required.

- Prepare 3 mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H<sub>2</sub>O).
- ✤ Press "RUN" and "HIGH" on fluid control panel.
- Install a tube of FACS Clean solution on the SIP and allow the cleaning solution to run for **10** minutes with the sample flow rate set to HI.
- ♦ Repeat step with BD<sup>TM</sup> FACSRinse solution and with Milli-Q  $H_2O$ .
- Push "Standby" button and leave tube with 1 ml of MQ Water on the sip.

**Note:** Please be sure in all the cases not to exceed the maximum allowed volume

of 2 ml in the tube.

### 10. Export FCS Data / Experiment

To save Experiment, right click on the Experiment Export > Experiment > Browse to choose the destination folder (D/year/AG/name).



Create a new folder and rename, then click Export > OK

		💽 Ехро	rt Experiments	
50514	✓ Ø Ø Ø Ø Ø Ø Ø Ø	Directory	Delete experiments after export  Directory Export Di(USER\Anatomy\Emily Pang\today)	Browse
		Experim	nent Da	te
		2015061	6 6/16	/15 4:09:01 PM
e: D:\USER\Anatomy\Emily Pang\today	Export			K Cancel
	So514 e: D:(USER(Anstomy(Emly Pang)today	avy V V T C C C C C C C C C C C C C C C C C	av Sosid Export Directory Experim 2015061	avr       Image: Constraint of the second seco

To save FCS files, right click on Experiment > Export > FCS Files



Select FCS 3.0 > OK > Browse to choose destination folder (D/year/AG/name).

Gated Events		
File Version O FCS2.0  O FCS3.0		
Parameter	Parameter Type	
FSC-A	O Linear ○Log ○ None	^
FSC-H	● Linear ○Log ○ None	
FSC-W	● Linear ○Log ○ None	
SSC-A	● Linear ◯ Log ◯ None	
SSC-H	● Linear ◯Log ◯ None	
ssc-w	● Linear ○Log ○ None	
FITC-A	● Linear ○ Log ○ None	
FITC-H	● Linear ○Log ○ None	
PE-A	●Linear ○Log ○None	
PE-H	Linear ○Log ○None	
APC-A	● Linear ○ Log ○ None	
	0.000	~

uments	and Settings\Adr	ninistrator\Deskti	op\user Br	owse	
	Save	Details>>	Cancel		8.

Create a new folder and rename > Choose Directory and save the file.

Look in:	🚞 user		· 🦻		
	Anatomy				
Mu Recent	Biochemistr	У			
Documents	Pathology				
123	SCRMC				
1					
Desktop					
Documents					
10					
20					
/ Computer					
	lage second				
v Network	File name:	C:\Documents and Settings\Administrator\Desktop\user		Choose Directory	
Distant	Files of type:	Directories	×	Cancel	

- Duplicate the experiment if needed again:
  - Select the experiment; right click and select dublicate without data. This retains the mask, the labeled samples and the stored cytometer settings
  - o It is not allowed to store more that 3 experiments without data
- Mandatory: Delete the measured experiment after having FCS data / experiment exported.
- Mandatory: remove your data (within 7 days) from the hardcopy drive D e.g. by transfer to (S/year/AG/name).

### 11. Log Out

To log out of FACS DIVA software, go to File > Log Out



### 12. Shut down

- Before you leave please be sure to switch the instrument off if required. Policy of keeping instruments "ON" or "OFF" depends on the time of the day:
  - **During the daytime**, we keep the analyzers **"ON" except** there is a gap between users longer than 3 hours;
  - If you finish your BD LSR-Fortessa experiment after 6 pm on the weekday or any time on the weekend and if the next person is not physically present at the site we request to switch "OFF" the BD LSR Fortessa and FACS Flow system.
- Please be sure to logoff Windows and shutdown the computer before you leave FCF Berg to avoid unnecessary charges.
- 13. Sign in the labbook (next to the computer) for documentation

*Note: Please report all the problems/concerns to FCF Berg Staff:* 

**During business hours** — get to Room 581/527 and tell the FCF Berg staff member.

**After hours** — e-mail to fcf-berg@med.uni-tuebingen.de; leave the note about the problem on the instrument's keyboard; proceed on step 9b from the above list.

- Empty Waste Tank Procedures (during measurement)
  - Make sure the flow cytometer is in **Standby** mode.



- Empty the waste tank.
- Add about 0.5L of clean into the waste tank
- Place the lid back to the waste tank.
- press restart on the FACS Flow supply system
- press **PRIME** on the cytometer
- Refill Flow Container Procedures (during measurement)
  - Make sure the flow cytometer is in Standby mode (see above).
  - Open the lid of the flow container
  - Replace by a new flow container
  - Close the lid
  - press restart on the FACS Flow supply system
  - permanently press PRIME (Pump 1 Fill) on the FACS Flow supply system until the light goes out
  - press **PRIME** on the cytometer