Northern Lights user manual



Basic user guide

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<u>Start-up</u>

NL has a 30 min warm up time. Timer starts after opening a template



 Power on the cytometer. Power button on the left-hand side of the cytometer.

Windows password: Welcome#1

- 2) Open the SpectroFlo software
- 3) Log in

Start typing your name in the username field; mouse-click to select user account Password: first letter of first name; entire last name; all lowercase

QC/startup account U: QC_startup | P: CytekNL

Run water during warm up

- 1) Click the [acquisition] icon on the welcome screen
- 2) Select the [Default] experiment layout
- 3) Remove the water tube from the sample probe
- 4) Load a tube with 2.5mL milliQ water
- 5) Set the flow rate [high]
- 6) Click on [start ▶] and run water as the cytometer warms up
- 7) (approx. 25 minutes).

when changing sample tubes, wait until the SIT flush is complete.





Spectroflo icon and PDF user manual





<u>Daily QC</u>

- 1) SpectroFlo QC Beads are in the fridge in 9-52 (chem prep)
 - a. Check if there is already a 5 mL tube made up (need >80 μL for a QC run)
 - b. If need to prepare a new bead tube, Vortex bead bottle and add one drop to 300 μL milliQ water in a 5 mL tube. Vortex tube.



2) Click on the QC tab in the blue ribbon, top right



- 3) Load 5 mL bead/milliQ tube on the cytometer
- 4) Check bead lot
 - a. Bead lot on the side of bead bottle
- 5) Click start
- 6) QC will take 5 minutes
 - a. After QC finishes, return bead bottle and bead/milliQ tube (if >100 μL) to fridge



New experiment

1) Click on the [acquisition] tab on the blue ribbon.



2) Close the wash experiment.

(X) to right of experiment name; to left of "WORKSHEETS"	
Iorning Wash Save As F Tube Group V Collapse All	
 3) Select [New] experiment layout ⊕ New 4) Enter exp name (YYYYMMDD_exp name) 5) Chaose parameters 	Library > Blue Laser
Expand library subgroup and double-click on fluorophore needed If not there, use the closest fluor	 Red Laser Click > to expand
Create New Experiment Fluorescent Tags Groups Markers Keywords Acquisition	
Name 20231115_testy mctestFace Enter exp name Description (optional) Library Expand to see fluorophores bue Laser > Bue Laser > Red Laser > Violet Laser > Fluorescent Proteins	3 Tags Added Selection BB515 APC BFP
 Viability cFluor Immunoprofiling Kit Tags 	

- 6) Click [next] (bottom right)
- 7) Add a reference group (single stain controls)

Fluorescent Tags	Groups				
Group	Tube	1: -	Collapse All	Debre	Reference Grou

8) Setup single stain controls

Set cells or beads.

Set [negative control] for fluors if these controls do not have an "internal negative population (ie, CD45 or cells that are 100% GFP+).

Define Unstained Contro	l(s) for Autofluorescence I	Extraction			Select if used	d beads	
Jame Jnstained			Control Type Cells	Defi	ne Additional Negativ	e Control(s) for Spil	llover Calcu
Fluorescent Tags Fluorescent Tag	Control Type	_	Label	Lot		Negative Contro	51
BFP	Cells	*					-
BB515	Cells	-	2 2				-
			10				

9) Add a new group for the FMOs and full stain samples

Create	New Ex	perir	
Fluorescent Tags	Groups	Marke	
			How many tubes to add when click on [+] group
Group	t Tube	1	
✓ <u>Add Gloup</u> 2023	1115_testy mcte	stFace	
✓ R	Reference Grou	р	
Π ^R	Unstained (Cells	5)	

 Group Collapse All 20231115_testy mctestFace Reference Group Unstained (Cells) Unstained (Cells) BFP (Cells) BB515 (Cells) APC (Cells) samples patient 001 	10) Set nar	nes for the tubes
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Unstained (Cells) Image: Rest of the second seco	¥ R	Reference Group
□R BFP (Cells) □R BB515 (Cells) □R APC (Cells) ▼ S samples samples	UR	Unstained (Cells)
Image: BB515 (Cells)	Ū٣	BFP (Cells)
Image: Constraint of the second se	UR	BB515 (Cells)
 samples patient 001 	Ū۴	APC (Cells)
U patient 001	√ S	samples
	U	patient 001

11) Click [next]

12) Set marker names

Click on the box at the experiment level (see below) to set the name for all tubes (in the image below, did not press [enter] yet, so it hasn't applied label to all.

Can also set labels at the group level

Will not appy to reference controls

Keitt Let Enter label here to apply to all tubes Groups BFP BB515 APC Name BFP BB515 CD45 • ● 20231115_testy metestFace CD45 • ● 20231115_testy metestFace CD45 • ● 10021115_testy metestFace CD45 • ● 100211115_testy metestFace CD45 • ● 100211115_testy metestFace CD45	Fi	uorescent Tags	Groups	Markers	Keywords	Acquisition		
Groups BFP BB515 APC eriment level • • • 20231115_testy mctestFace • CD45 • • • • Reference Group • • • • • Reference Group • • • • • • • • • • • • • • • • • • •		🧨 Edit Lot					Enter	label here to to all tubes
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Group level samples U patient 001			PC (Cells)		N/A	N/A		
1 patient 001	Group level	√ ि s s	amples					
		q U	atient 001					

- 13) Click next
- 14) Keywords (can leave blank)
- 15) Click [next]
- 16) Set stopping event number, volume, or time.

Again, can set at a global level, group level, or individual tubes

Name	Worksheet	Stopping Gate	Storage Gate		Events To Record
20231115_testy mctestFace	Default Raw Worksheet (Raw) 👻	All Events	✓ All Events	Ŧ	5,000
✓ Reference Group	Default Raw Worksheet (Raw) 💌	All Events	✓ All Events	•	5,000
Unstained (Cells)	Default Raw Worksheet (Raw) 💌	All Events	✓ All Events	*	5,000
BFP (Cells)	Default Raw Worksheet (Raw) 👻	All Events	✓ All Events	-	5,000
BB515 (Cells)	Default Raw Worksheet (Raw) 🔻	All Events	✓ All Events	•	5,000
APC (Cells)	Default Raw Worksheet (Raw) 🔻	All Events	✓ All Events	*	5,000
✓ samples	Default Raw Worksheet (Raw) 💌	All Events	✓ All Events	•	5,000
patient 001	Default Raw Worksheet (Raw) 🔻	All Events	 All Events 	*	5,000

17) Click [save and open]



Sample acquisition

Final protocol checks

- Flow rate (can not change mid run; requires [pause]
- User settings (loads fluorescence and scatter gains and threshold)
- A DO NOT change individual channel gains. If signal too high or low, change via the "all channel %" setting.

Ballpark user-settings (derived from cytek assay settings)

	leuk (human)	Cell lines
FSC	50	20
SSC (violet / blue)	145 / 160	60 / 60
Threshold	FSC, 350,000	FSC, 350,000
V1	45	20
B1	1090	50
R1	80	20

Use the all channel % option to set V1, B1 and R1 to the numbers above. This will also set the other channels V2-V16, B2-B14, R2-R8 to the appropriate gain (relative to % change).

Changing 'all channel %', increases or decreases all the gains of that laser line by the number (percentage) entered. The % value is relative to the loaded user settings. So, If you save these settings, the % value will be set to 0, with any changes from then being applied to the newly saved gains.

Instrument Co	ontrol	
User Settings:	CytekAssaySetting (Cytek)	
GAIN THRESH	CytekAssaySetting (Cytek)	
	LyseNoWashAssaySetting (Cytek)	Instrument Centrel
449	BC multicheck	Instrument control
Violet B	CytekAssaySetting	User Settings: BC multicheck
V1 V2 153 \$ 22	CytekAssaySetting_Copy_002	
V6 V7 154 20	Default	GAIN THRESHOLD SIGNAL LASERS
V11 V12 211 1 19	k562-bradley U LyseNoWashAssaySetting	FSC SSC 405nm SSC-B 488nm
V16 232 \$	Water settings	50 \$ 145 \$ 161 \$
Choosing p	redefined user settings	Setting FSC and SSC

GAIN	THRESHOLD	SIGNAL	LASERS					
FSC	SSC	SSC-B						
50 1	145 🗘	161 🗘						
Violet	Blue	Red						
		V 3	V4	V5				
46 ‡	69 ‡	55 ‡	40 ‡	55 ‡	GAIN	THRESHOLD	SIG	NAL LASERS
V6	V7	V8	V9	V10			-	
46 ‡	61 ‡	85 ‡	64 ‡	73 ‡	Thrachala	Operator:	Or O	And
V11	V12	V13	V14	V15	meshoic	Operator.	010	Anu
63 ‡	57 🗘	51 🗘	61 ‡	83 ‡				
V16				-17 - 18 	Channe	1		Threshold
70 🌲								
All Chann	els %:			0 2	FSC		*	350,000
	Chan	ge all gain	s for			Threshold sett	ings	
	the s	selected la	iser					

1) Load unstained sample, this will be the first tube in the group.

If you want, you can load a full stain tube to check if the fluorescence signal is on scale. Then switch back to the unstained to record data for that tube.

2) Press [Start ▶]

Make sure that scatter and snake looks ok (ballpark: set lowest channel (of each laser near 0)



Scatter and snake for unstained PBMCs

3) Press [Record] to save data for that tube

Once finished, the selected tube will go down the line of the group. You can manually change tubes by left-clicking on them.



when changing sample tubes, wait until the SIT flush is complete.

4) Run/record all reference controls and FMO/full-stains

<u>Unmixing</u>

1) C	lick unmix
U	20231113_multicheck
••	Save Save As Edit Unmix
*ប	🚰 Tube 📑 Gr Unmix Experiment pse All
	> R Reference Group
	✓ S amples

2) In the new window, click the auto-fluorescence button.

ct Controls Ider	ntify Positive/Negative	Populations QC	Control
NSTAINED CONTRO	DLS		
) Use Control from Lib	rary		Ŧ
Use Control from Exp	periment Reference G	roup - Unstained (Cells	;)
Name		Control Type	
Reference Group - U	nstained (Cells)	Cells	
Reference Group - U	nstained (Cells)	Cells	
Reference Group - Un TAINED CONTROLS	Fluorescent Tag BB515	Cells Control BB515 (Cells)	
Reference Group - Ui TAINED CONTROLS From Library	Fluorescent Tag BB515 PE	Cells Control BB515 (Cells) PE (Cells)	•
Reference Group - Ui TAINED CONTROLS	Fluorescent Tag BB515 PE PE-Cy5	Cells Control BB515 (Cells) PE (Cells) PE-Cy5 (Cells)	
Reference Group - U	Fluorescent Tag BB515 PE PE-Cy5 BB790	Cells Control BB515 (Cells) PE (Cells) PE-Cy5 (Cells) BB790 (Cells)	

3) [next]

4) Set the scatter gate appropriately and then set the positive and negative populations for each fluorophore.

FSC/SSC: Tight selection around cells of interest. Positive peak: Mode and greater, or brightest 500-1000 events Negative peak:

You can manually change the channel used for setting the gates by dragging the "error bar" on the snake graph to another channel



5) Select the [live unmix] button to apply unmixing to this experiment (the non reference group samples).

			\rightarrow
Cancel	Next \rightarrow	Create New Unmixed Experiment	Live Unmix

Exporting data

Export files to open in FCS express or flowJo.

- 1) Right click sample group
- 2) Select [export FCS]
- 3) Save on D:\ in your folder
- 4) Raw and unmixed files exported. Open the unmixed file in analysis software



1.0M

2.0M

DATA (D:) > Bradl	ey Chereda → 20231113
	Name
	20231113_multicheck-samples-FMO hla-dr.fcs
	20231113_multicheck-samples-FMO hla-dr_Unmixed.fcs
	20231113_multicheck-samples-full stain.fcs
	20231113_multicheck-samples-full stain_Unmixed.fcs

<u>Analysis</u>

You can export the unmixed data to FCS express or plot data in spectroFlo.

1) Create a new mixed worksheet

Clicking the (+) to the left of the [REPORTS] Select [new unmixed]



2) Create plots and gates

To change the parameter, right click the plot axes

To set a gate to a plot, right click the plot > properties > set the gate

The properties window also allows bi-ex limits, dot size.

			Dot plot		plot		Interval gate			Bin	Binary gate		Edit spi		spil	lover				
	heatmap		Square gate		ate		Hinged quad		d	Gate hierarc		archy	save			PDF expor		ort		
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			ł	nisto	Poly	gate		(quadra	ant	S	tatist	ics [·]	Text bo	х		Sav	e as		
						Ellij	ose g	ate												



Standby wash

- 1) Prepare 2 tubes
 - a. 2.5mL sodium hyperchlorite
 - b. 2.5mL water
- 2) Click the third icon on the left (cytometer)
- 3) Click [clean flow cell]
- 4) Follow on-screen instructions.
- 5) Log out of SpectroFlo



<u>Shutdown</u>

- Click the third icon on the left (cytometer) > fluidics shutdown
- 2) Prepare 3 tubes (2.5 mL):
 - a. Bleach (1:1 bleach : water)
 - b. Water
 - c. Contrad (1:3 contrad : water)
 - d. Water, can refill the second tube.
- 3) Follow on screen instructions
- When finished, software will instruct to power down cytometer (power button on side of machine)
- 5) Log out of SpectroFlo
- 6) Leave PC on, it will go into standby.



Sheath and waste

Waste tank full

First, empty decontaminated tank (on preparation table)

- 1) Using the biocan-holder, take the waste tank to the chemical preparation room.
- 2) Turn on water tap
- 3) Unscrew waste tank lid and decant into the sink.
- 4) Return to 9-50 with the empty waste tank and biocan-holder.

Second, decontaminate the now full waste tank

- 5) Unplug fluidics line on the waste tank lid (quick connect)
- 6) Unplug electronics cable from the cytometer body (black wire)
- 7) Move the waste tank to the preparation table behind the cytometer, put it in the biocan holder.
- 8) Add 350 mL sodium hypochlorite to decontaminate waste
 - /!\ Do NOT throw out hypochlorite bottles; they will be reused.

Connect empty waste tank to cytometer

- 9) Put empty waste tank in the holder
- 10) Attach the fluidics and electronics lines.
- 11) Resume your cytometer run.





Decontaminating waste tank, placed in the biocan-holder

Sheath tank empty

- 1) Disconnect fluidics line from the sheath tank lid.
- 2) Remove empty sheath tank from the holder
- 3) install the full sheath tank in the holder and connect fluidics line.
- 4) Refill empty sheath tank. Use the chemical preparation room Satorius system.
- 5) Unscrew lid and fill tank with 3.5 L of milliQ water.

Biocan holder available to aid in transporting between the chem prep room and 9-50.

6) 6/ Place the newly filled sheath tank to the left of the in-use one.



(left) place filled sheath tank here (right) installed shealth tank