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START UP PROCEDURE

Is the yellow laser required for this experiment?

- If so, it needs to be turned on separately (see page 3)
- The corresponding QC protocol then needs to be run to include the yellow laser (Align 4-laser)

Turning on the Gallios.

- Turn Gallios on using icon on desktop
 - 10-15 min warm up time
 - Green light on front panel indicates system ready
- Open the Gallios Software on the desktop
 - Login to your profile
 - Connect



Data management

The Gallios produces LMD files. Data are automatically backed up to the RDS cloud server each morning at 9am. If you need the files the same day, you must organize with staff beforehand.

The computer must be logged into Windows for the backup to work successfully.

YELLOW LASER

- 561nm laser
- Excellent for PE and PE tandems
- Uses the same pinholes as the blue, 488nm laser (co-linear)
- Little to no compensation required between FITC and PE
- Can choose to have laser on or off

Turning yellow laser ON

- Turn on the Gallios, using the desktop icon
- Turn the key to the ON position (|)
- Hold down the green 'LASER ON' for 3 seconds
- Once the top light in the 'Laser Emission' section has stopped blinking (after approximately 30 seconds), the laser is on and ready for use
- Log into software and proceed to running QC



QC info

- If you are the first user of the day, make sure to choose the 4 laser QC application when running QC
- If the Gallios was used earlier in the day, you will have to run QC again if the 3 laser application was run.

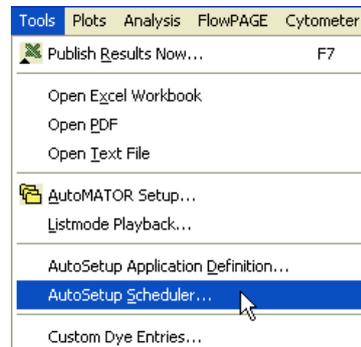
Turning yellow laser OFF

- It is the users' responsibility to turn it off correctly after machine shutdown/standby
- Hold down the red 'LASER OFF' button for 3 seconds until the Laser Emission lights go out
- Then turn the key back to the OFF position (O)
- The red error button will blink when the cytometer is on and the yellow laser is not

DAILY QC

- Prime the Gallios  (“white martian” button) to clear the fluidic lines after being off overnight.

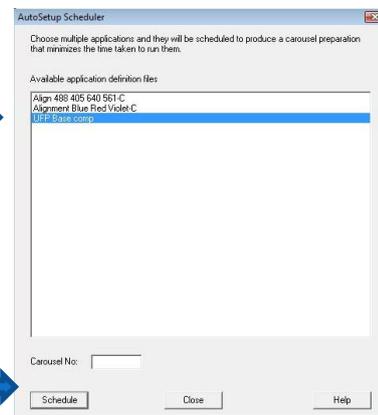
- Log in to your user profile
- Schedule the QC run
 - Tools
 - Autosetup Scheduler



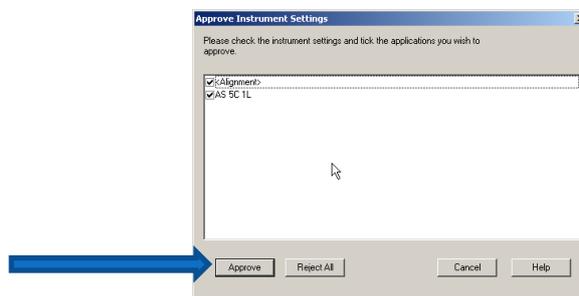
- Choose the Application that matches the laser setup. 3 laser (yellow laser **off**) or 4 laser (yellow laser **on**)



- Enter the carousel number and click ‘Schedule’



- Put the 5mL flow tube containing FlowCheck Pro beads into the correct position as indicated on the ‘Carousel Load Report’ and select ‘Run’
 - QC tube generally found in front of keyboard. Or,
 - The stock bottle is in the under-bench fridge to the right of the Gallios. Squeeze $\approx 200\mu\text{L}$ straight into a 5mL flow tube
- Once the run is finished and passed, click ‘finish’ and ‘Approve’ the run



- Record that you have run QC in the Log Book on the desktop
 - On the desktop is a text file named “QC Log Book”
 - Each QC run needs to be recorded
 - Include any notes about performance

QC Troubleshooting

Symptom: Can not schedule run

Solution: did not clear file name tags in [Workspace preferences] in last run. Click floppy-disk icon in worklist window (see right). Clear all except for [run number]

Sample ID 3	Sample ID 4	LMD Filename
		00062581.LMD
		00062582.LMD

Symptom: 3 or 4 of the PMTs too bright (FL2, FL3, FL4 and FL5)

Solution: Yellow laser ON in 3 laser protocol

Symptom: FL2, FL3, FL4 and FL5 very dim

Solution: Yellow laser OFF in 4 laser protocol

Symptom: FL4 fails QC

Solution: Could mean the incorrect filter has been left in; please find a staff member for assistance.

LJ plots aren't currently functional:

- Previous performance in QC by the Gallios can also be checked by opening up the Levey Jennings plots by clicking the graph button on the home screen 
- From the 'Template' menu select the appropriate matching template for the protocol you want to analyse (either 4 or 3 laser) as each will be different
- This screen will be generated



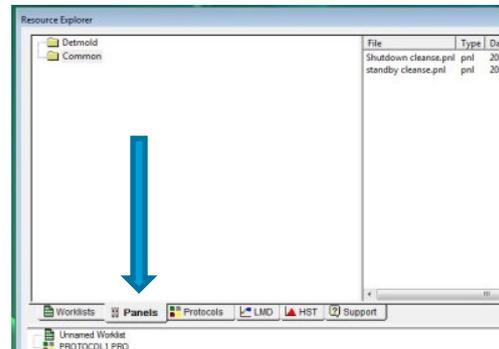
- QC runs from previous days can be analysed and any trends or failures can be noted
- In this screen there is also the opportunity to record any notes about the QC runs
 - Simply click on the appropriate dot, add comment and SAVE
 - Particularly useful if something breaks down and we can look for trends or for reviewing performance

GALLIOS STANDBY PROCEDURE

The Gallios must be washed after your session. A stand by wash is to be run when there is another user following for that day. The last user of the day will need to run **Gallios Shutdown Procedure** (next section).

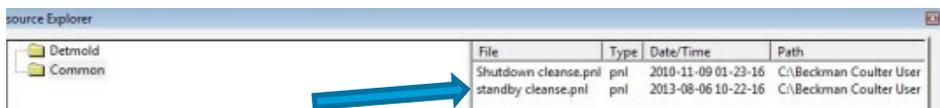
The stand by wash is run from within a user profile.

1) In the resource explorer window, click on Panels.



There will be 2 folders –Acquisition and Common.

2) Select **Common**



3) Load the appropriate cleaning carousel.

4) Click on and drag standby cleanse.pnl into the Worklist Manager (green and white list window usually found on right screen at bottom)

5) Enter the carousel number (cleaning carousel: 1) and then press the play button to begin the clean.

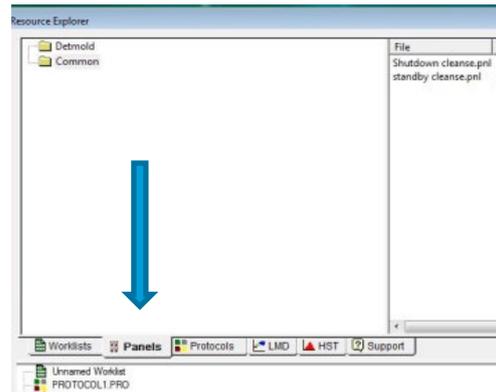
*Users must stay whilst the wash is running to make sure it doesn't stall or error.
Any time spent logged in to a user profile is "charged time".*

6) Once completed successfully, log out of the user profile.

GALLIOS SHUTDOWN PROCEDURE

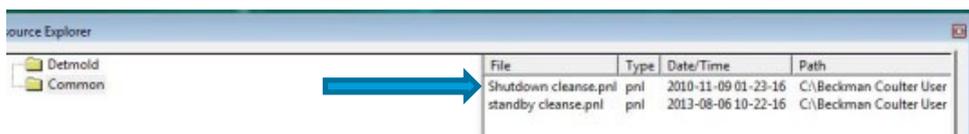
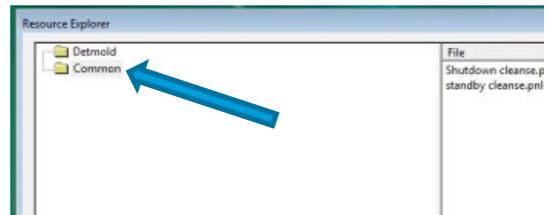
The shutdown wash is run from your login.

- 1) In the resource explorer window, click on Panels.



There will be 2 folders –Acquisition and Common.

- 2) Select **Common**



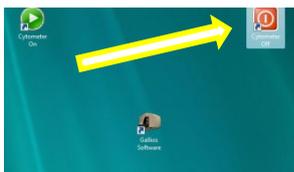
- 3) Load the appropriate cleaning carousel.
- 4) Click on and drag Shutdown cleanse.pnl into the Worklist Manager (green and white list window usually found on right screen at bottom)
- 5) Enter the carousel number (cleaning carousel: 1) and then press the play button to begin the clean.

Users must stay whilst the wash is running to make sure it doesn't stall or error.

- 6) Once completed, the analyser will show “awaiting sample”. Press the Cleanse button:



- 7) The analyser will show “Idle” in the bottom status area once finished. Then exit the Gallios software.



Press the Cytometer Off button on the desktop to turn off the Gallios.

Leave the PC logged into windows. This is required for the data backup to occur each morning.

If the yellow laser was in use, it will need to be turned off correctly. The laser can be turned off prior to/during/following the shut down procedure. See *Yellow Laser* page 3.

Making Protocols

Plots

- Histogram or density plots
- Make parameter display choices upon creation
- To change parameter later or display percentages
 - Right click on plot
 - Format plot



Gates

-  Polygon gate
 -  Rectangle gate
 -  Quadrant gate
 -  Linear region graph
 -  Multiple linear region graph
- } Dot/density plot
- } histogram

Save protocol

- Press when you make any changes to the protocol either gating, voltage or flow rate.
- Insurance against computer crashes

Making a worklist:

- Sample ID 1: Date (in format: yyyyMMdd) ***mandatory***
- Sample ID 2: Cell line/type
- Sample ID 3: Fluorophore
- Sample ID 4: treatment / titration / etc.

You can use whichever labeling strategy you wish for IDs 2 -4, the above were just examples. However, ID 1 must be the date in the stated format.

Setting LMD file name:

The default file name is a sequential run number. If you want to include other tags, click the [save] icon in the worklist window next to [LMD filename]

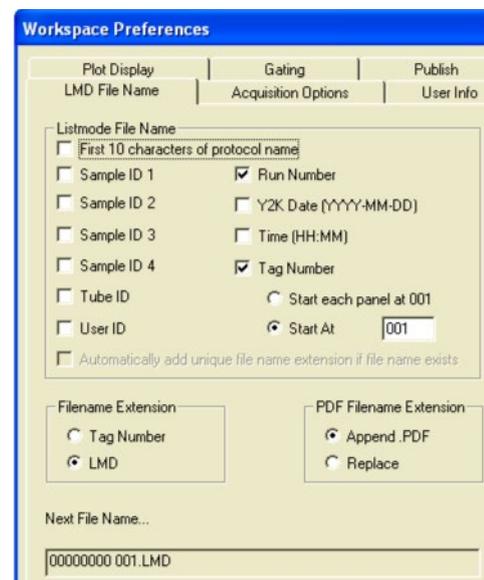
ID 1	Sample ID 2	Sample ID 3	Sample ID 4	LMD Filename
				00062581.LMD
				00062582.LMD

You must include the run number and [Sample ID 1].

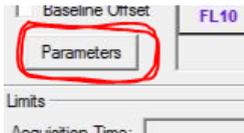
Other tags can be used to easily distinguish the data files.

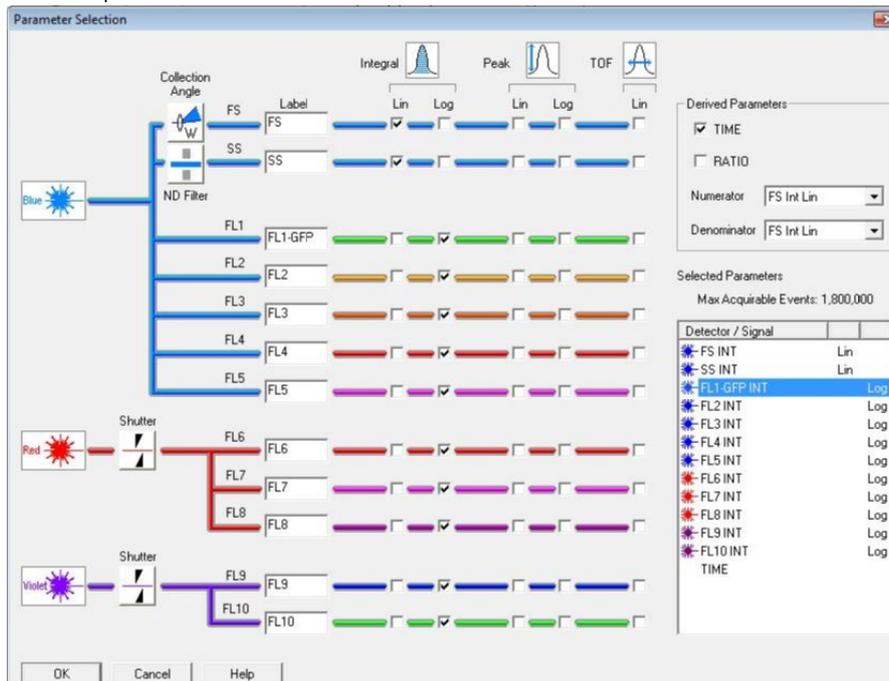


Before the clean carousel, you must reset the checkboxes to only have [Run Number] selected. Otherwise you wont be able to schedule QC on your next run



Parameter setup window (adding / removing params)

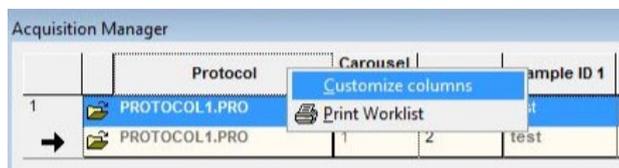
- Open the cytometer control window: 
- Click on “Parameters” (left hand side) 
- From here you can:
 - Enable: integral (area), peak (height), TOF (time of flight; or width)
 - Disable any parameters (checkbox) or lasers (toggle “shutter”)
 - Lin or Log for a given parameter
 - Alter forward scatter / side scatter angle / filters (advanced; recommend not to change these settings unless you need to)
 -  Editing the label here will not carry over to your data file. See the next section to label parameters.



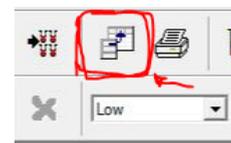
Parameter naming

Edit the parameter names in the acquisition manager window

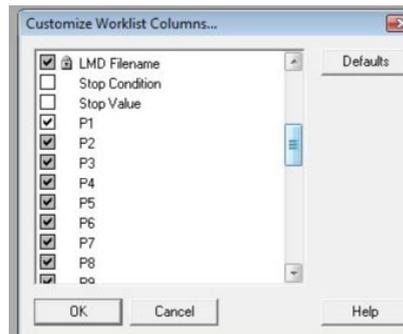
- Right click a column and select customize columns



or press:



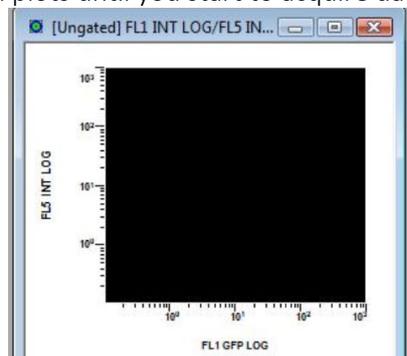
- Select P1 (which will select the other parameters) and click OK.



- You will see all the parameters in the worklist. Edit the parameters here

Protocol	P1	P2	P3	P4
PRO	FS INT LIN	SS INT LIN	FL1 GFP LOG	FL2 INT LOG

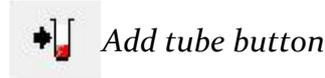
- You will not see changes on plots until you start to acquire data.



- You can increase the size of the axes' text via right click, format plot and go into the font tab.

Running samples (from scratch)

- 1/ Load carousel
- 2/ Create worklist (naming each tube correctly)
- 3/ Setup parameters and initial cytometer settings
- 4/ Draw plots
- 5/ (optional) create flowPage (next section, page 13)
- 6/ Save protocol
- 7/ Start sample
- 8/ Adjust voltages and gains
- 8a/ Save protocol's final changes
- 9/ Begin recording data



Add tube button

Creating a worklist

- Click "File" > "New" > "Worklist" to clear the current worklist
- Add the number of tubes you have loaded on the carousel
- Label tubes appropriately via "sample IDs"
 - Note: whatever you name the first tube will be replicated down for all the other tubes. This is useful for the date (sample ID1; yyyyymmdd) and maybe other labels, but make sure you check each tube's labelling.
- Setup LMD file save names (see page 8)

Setup parameters and cytometer



Cytometer control button

- See previous section on setting up parameters
- Open the cytometer control window (you should probably keep this open throughout the acquisition)

A / Setup mode. When checked: auto record is disabled. Data will be displayed so you can edit voltages and gains. Uncheck when you are ready to record data

B / set the number of events that will be recorded

C / set the stopping gate

D / slider for changing voltages. Select the FL to change (here FL1 is selected) and use the slider to alter the voltage. Clicking above or below the slider will change the voltage by 10. You can also type numbers in the selected box.

E / Descr. (discriminator) is the parameter's threshold value

	Label	Discr.	Volts	Gain
	FS	100	250	1.0
	SS	OFF	250	1.0
	FL1	OFF	250	1.0
	FL2	OFF	250	1.0
	FL3	OFF	250	1.0
	FL4	OFF	250	1.0
	FL5	OFF	250	1.0
	FL6	OFF	250	1.0
	FL7	OFF	250	1.0
	FL8	OFF	250	1.0
	FL9	OFF	250	1.0
	FL10	OFF	250	1.0

Limits

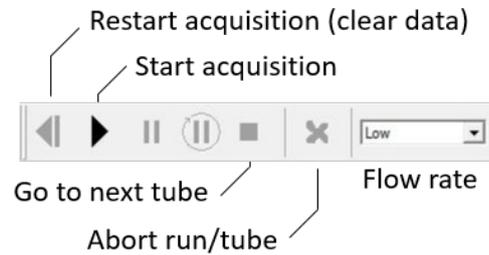
Acquisition Time: 300 Maximum Events: 100000

Elapsed Time: 300 Live Gate: Ungated

Max Acquirable Events: 1,800,000

Start sample | adjust voltages | record

- Remember to select “setup mode” in cytometer settings or the computer will automatically begin recording data



- Press play to start the run
- Flow rate: low, medium, high (this setting will be saved with the protocol)
- When setting voltages, gains, discriminators: use the “restart acqu” button to restart data acquisition, to get a better idea of what the change did.
-  **SAVE THE PROTOCOL** the latest saved settings or default settings (if you never saved the protocol at all!) are loaded when starting the next tube
- Deselect “setup mode” to begin recording data.

Creating a FlowPage (PDF template)

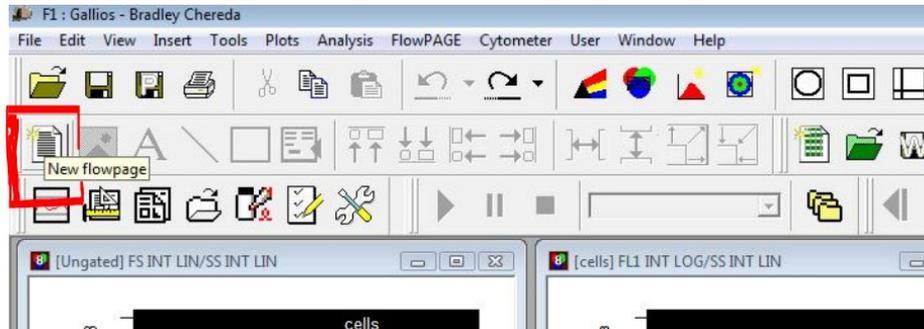
A flowPage is an A4 layout of flow plots or statistics. They are linked to a protocol and generated after each sample is recorded. You can configure one after you setup all your plots and statistic items in a protocol.

Inserting a flowPage

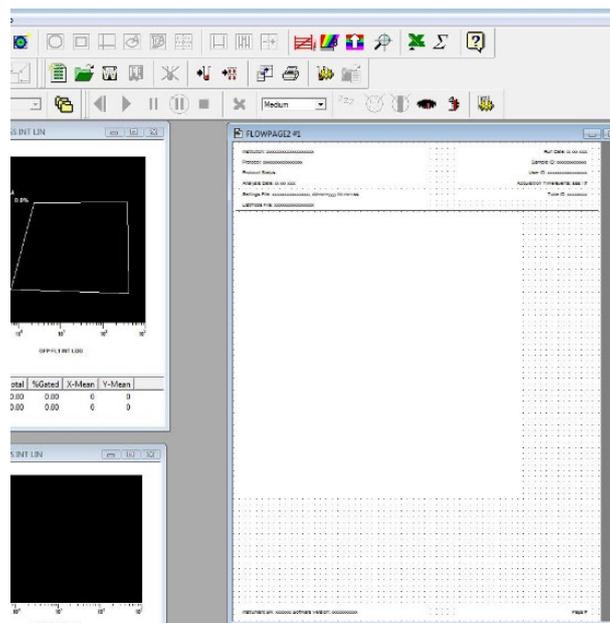
Click the [new flowPage button]



New FlowPage button

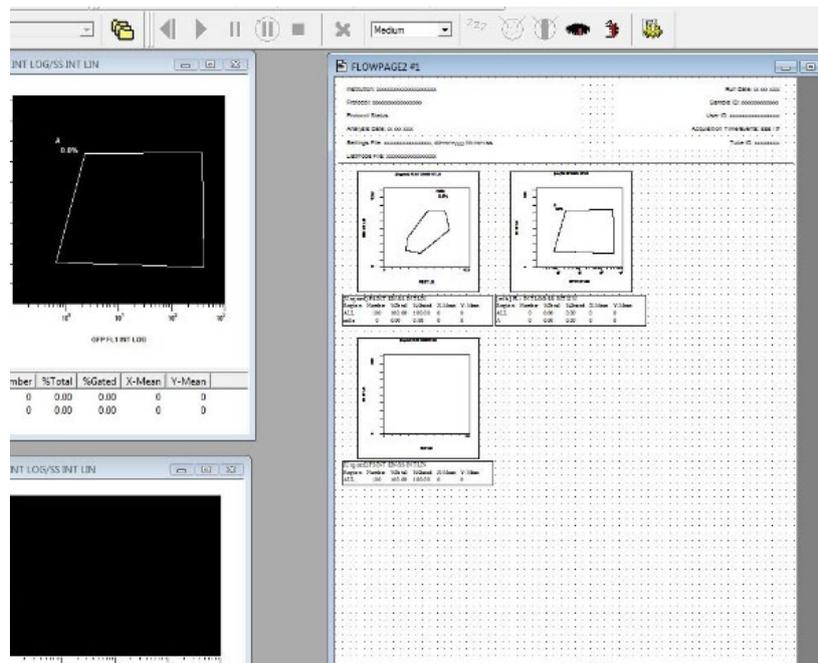


The flowPage will appear as in its own window/frame



Plots within the protocol can be added to the flowPage

- Click an ungated black area of a plot
- Drag onto the FlowPage window.

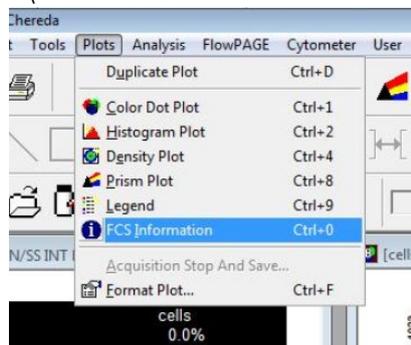


In the example above, the three plots in the flowPage will be saved as a PDF. The header includes date, protocol name, sample ID 1 and filename.

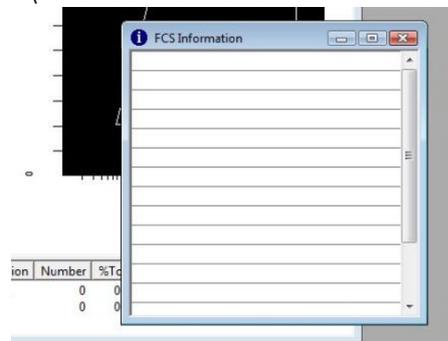
Add table to display SampleIDs

Adding this table will show all of the sample IDs to distinguish what sample the PDF is referring to.

1\ Plots > FCS information



2\ The FCS information table will be displayed



3\ Right click the FCS information table

- Select [format plot]
- Select [FCS] tab
- Select \$Date, @SampleID1, @SampleID2, @SampleID3, @SampleID4
- Click OK.

4\ Drag table onto FlowPage

5\ Minimize the FCS information window so it isn't in the way

6\ To edit the FCS information object in the flowPage, right click the object and select [Properties]

7\ Save the protocol or save-protocol-as

Enable saving the FlowPage PDF for each sample

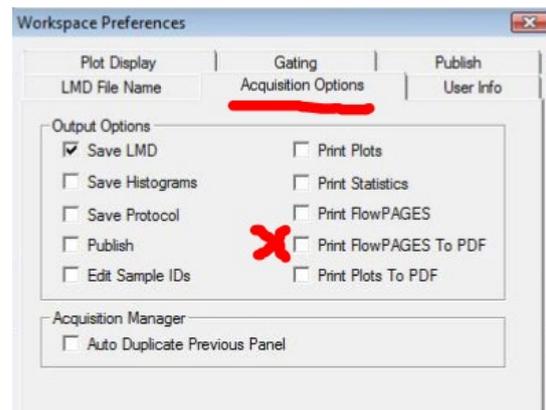
1\ Open workspace preferences (Save icon in the worklist window)

ID 1	Sample ID 2	Sample ID 3	Sample ID 4	LMD Filename
				00062581.LMD
				00062582.LMD

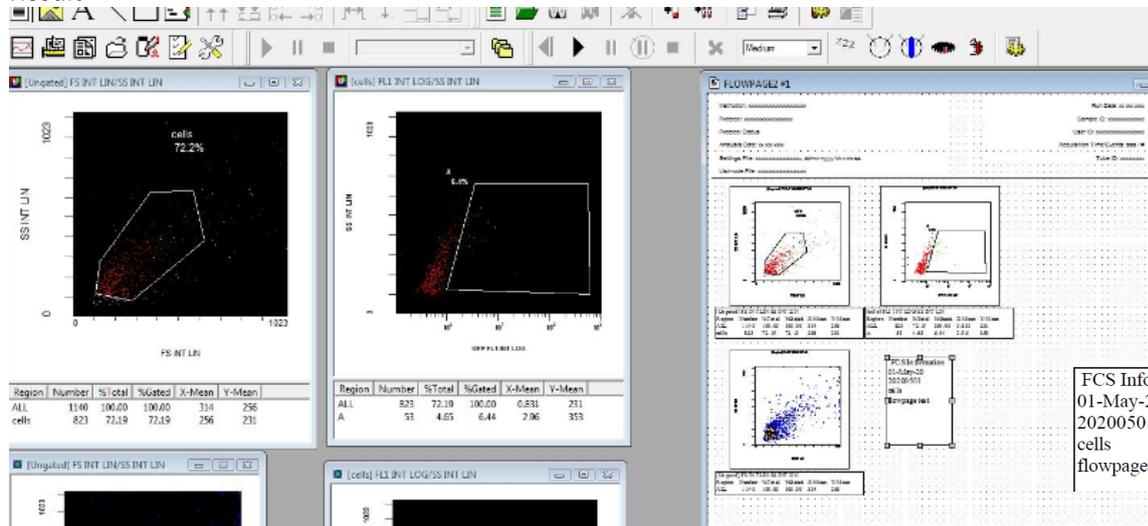
2\ Select the [Acquisition Options] tab

3\ Select the tickbox [print flowPAGES to PDF]

4\ Click OK



Result



PDFs are saved (cloud location):

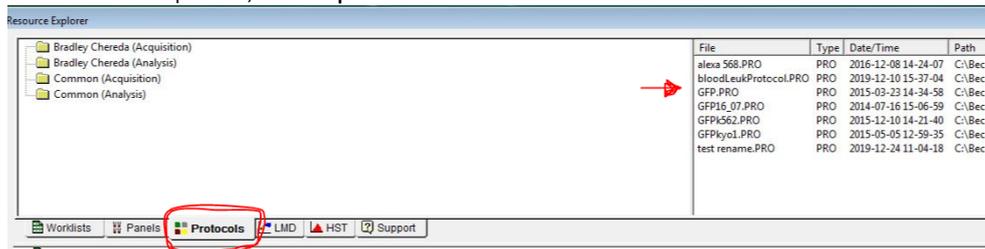
CCB Cytometry > Gallios > Data > username > PDF

Running samples (using a pre-existing protocol)

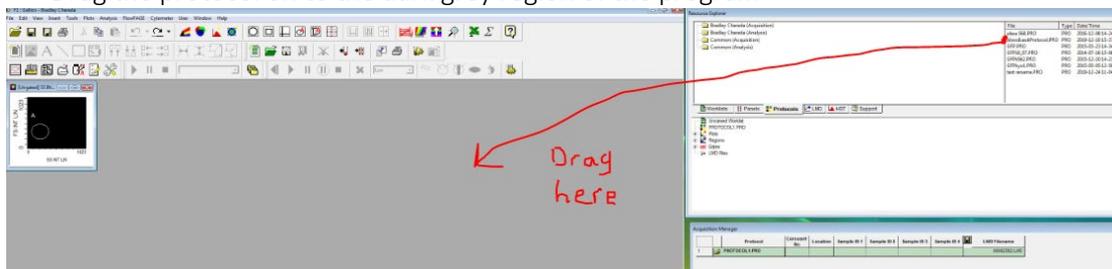
- 1/ Load carousel
- 2/ Drag protocol on grey part of software
- 3/ Create worklist (naming each tube correctly)
- 4/ Check parameters and initial cytometer settings
- 5/ Start sample
- 6/ Adjust voltages and gains
- 6a/ Save protocol's final changes
- 7/ Begin recording data

Drag protocol from protocol list

- In resource explorer; select **protocols** tab



- Your saved protocols will be listed on the right
- Drag the protocol on to the dark grey region of the program



- It will ask to save the previous protocol. If it was a legit protocol you made changes to (and want to save), press the Y key, otherwise press the N key.
- The protocol will loaded in the worklist, along with its plots and cytometer settings.

Check parameters / cytometer settings

- Make sure the parameters are consistent with what they were last time

Start sample

- Have "setup mode" selected (cytometer settings) to visualise the data and confirm voltages are set correctly.
- Change voltages if required and save protocol.
- Deselect "setup mode" to record data