FORTESSA BASIC USER GUIDE

CCB cytometry

Updated August 2024

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Fortessa Cheatsheet

Turn on

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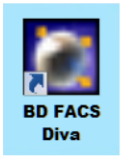
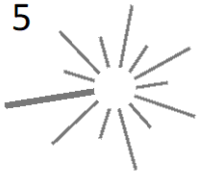
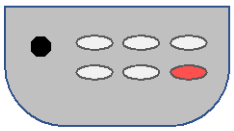
3  4 

5 

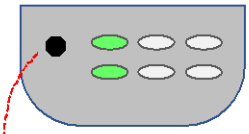
Turn Lasers on

CS&T

* Run CS&T if first user of the day. Subsequent users: check if CS&T was performed
* All Lasers must be turned on for CS&T



Prime x2

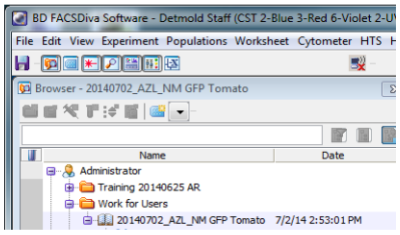


Run (low)

CS&T

Fine adjust: turn fully clock-wires, then dial back 6 turns

Diva



* Create / duplicate experiment
* Experiment name: **YYYYMMDD**_BC_etc...
  Must be in YMD format for data archiving
* Create tubes and compensation controls

Acquire

* Cytometer in [run] mode before loading tubes
* Before recording data, check cytometer voltages are suitable for today's run
* Acquire data for your samples
* Changing waste tank / sheath carton: see manual (blue folder or PC-desktop)
* Batch-process: Export PDFs (if required)

Wash / End of run

* **Standby:** there is another booking today
* **Shutdown:** last booking for today

	Standby wash	Shutdown wash
Bleach	2 min each **Hi** flow rate	5 min each **Hi** flow rate
FACSclean		
Water		
Lasers	next user start >30 min Turn off	Turn off
Standby	Put machine in standby	
Power off Fortessa	No	Yes

Leave computer logged into Windows or data-backup wont occur

START UP PROCEDURE

- If PC turned off, turn on before you turn on the cytometer

  Windows login Username Operator Passeord muse42

1. Open BD coherent software (don't turn lasers on yet)
2. Turn on cytometer

   Green button on right hand side of instrument

   Fortessa takes 60 seconds to communicate with PC. Do not start lasers or FACSdiva before then, or you may get a connection/laser error.
3. Press prime button on the front panel; wait for prime light to turn off; and press it again
4. Open FACS Diva software

   Login: select user name from dropdown list

   Password (all lower case) : first letter of first name and full last name

   Check caps lock is not on

5. Turn lasers on

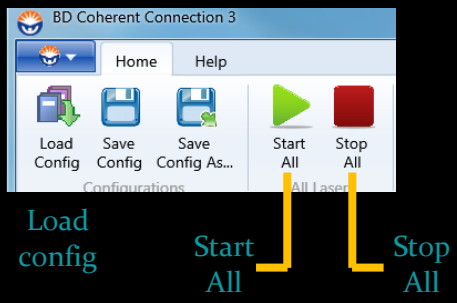
LASER START UP

- All 5 lasers are controlled by Coherent software.
- All lasers must be on for CS/T
- Blue (488nm) laser must be on at all times; for FSC/SSC.
- If your experiment does not utilize a laser, turn it off to conserve its lifespan.
- BD engineer has recommended not to turn off/on frequently. So only turn off lasers if next user will be in > 30 min.

1. Open BD Coherent Connection 4 software
2. The tiles for each laser will load after 30 seconds.
3. Click on [load config]
4. Select: [July 2024 5 lasers] > click open
5. Click on [start all] / or individually start lasers you need
6. Laser warm up: 1 minute
7. Make sure red dials indicate all lasers are on

   There is a bug where lasers turn off after 40 seconds.

   If this occurs, restart the Coherent software.
8. Cytometer ready for use

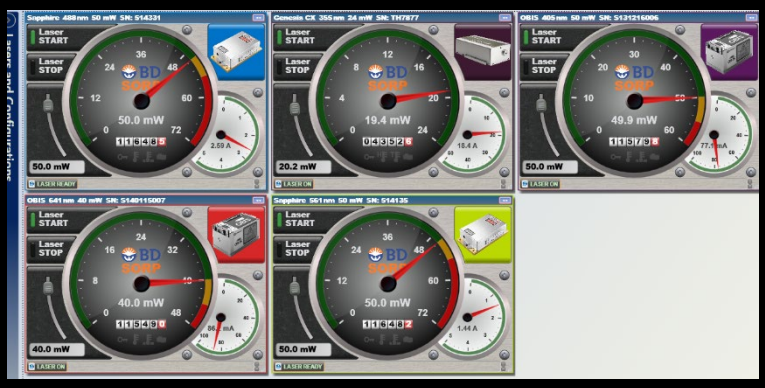


LASER SHUTDOWN

If End of day, or

Next booking starts > 30 min

1. Click [Stop All]



AVOIDING CONNECTION ISSUES

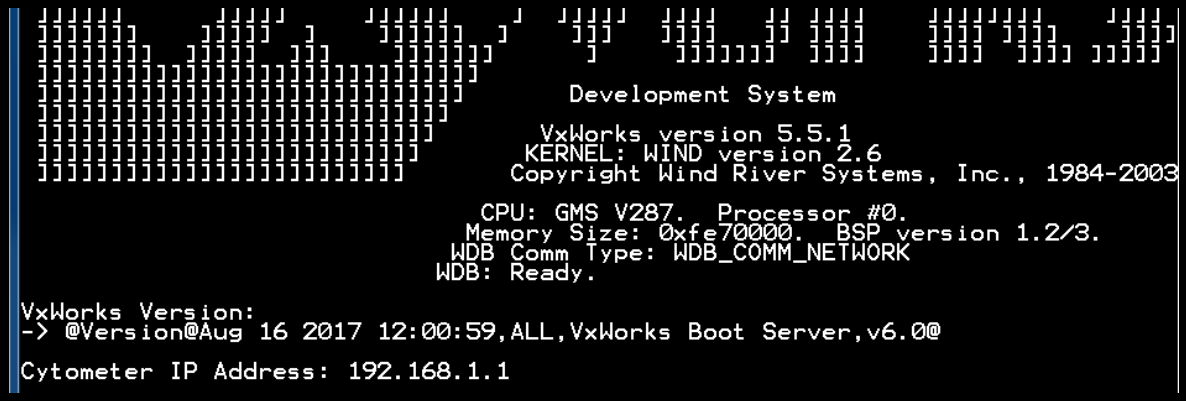
Logging into FACSDiva too soon after powering on the Fortessa can cause connection issues.

You can use the HyperTerminal app to monitor communication between the Fortessa and PC.

1/ Start HyperTerminal before turning on Fortessa

2/ Approx 15 seconds after powering on Fortessa, you should see some output.

3/ When you see the ASCII "WxWorks" image and "cytometer IP address 192.168.1.1, you can log into FACSDiva



CYTOMETER FAILS TO CONNECT

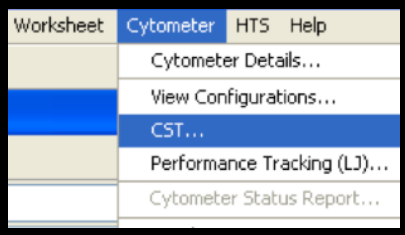
You will have to restart the cytometer

1. Log out of FACSdiva
2. Turn off Lasers
3. Turn off the Fortessa (green power button)
4. Wait 2 minutes
5. Turn on Fortessa (Fortessa-PC communication takes ≈ 1 minute)
6. Log into Diva and check if it will connect
7. Even if it says "disconnected" give it 15-30 seconds, sometimes it will try again and will connect
8. Turn on Lasers – do this after successful connection to avoid repeated power cycles of the laser.

If the above didn't work, you will need to restart the cytometer and PC

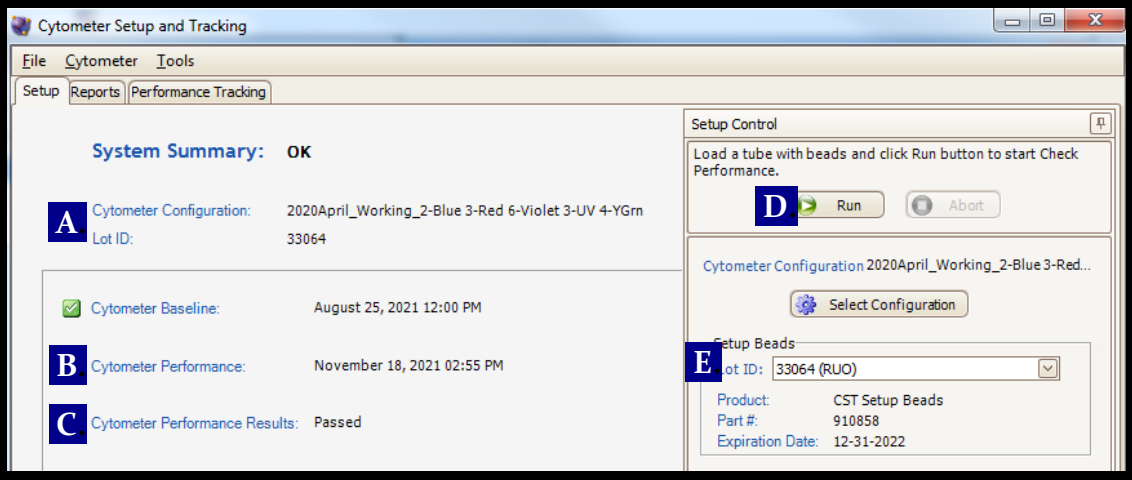
- Follow steps 1-3 above.
- Turn off the computer (shutdown, not restart – can crash when restarting)
- Step 4
- Turn on PC, and log in (username: operator)
- Continue from step 5 above

RUNNING CST –INSTRUMENT QC



- **Ensure all Lasers are turned on**
- On the front panel of the cytometer:
  - Turn fine adjustment dial fully clock-wires, then 6 rotations counter clock-wires
  - Press [Run] and [LO]
- In FACSDiva: from the [Cytometer] menu select [CST]
- Check if CST was run today **.B.** if not, need to run now.
- Make up CST beads (need to be made up fresh every day)
  - Beads and pre-filled PBS tubes (yellow rack) are found in the fridge next to the spill kit.
  - Vortex bead tube (5 seconds) whilst laying on its side, then squeeze 1 drop into prefilled tube (350µl of 1 x PBS)
  - Make sure that the lot number on the bead tube (not box) matches the lot ID in FACSDiva **.E.**
- Remove the O/N or water tube and load the 5mL PBS/bead tube
- [in FACSDiva] press Run **.D.**
  - CST Takes about 10 minutes to run
- Exit CST by pressing the red X at the top right-hand side of the window to return to the main cytometer window
- You are now ready to proceed with your experiment

.A. Current config: 2020April_Working 2-blue 3-red 6-violet 4-YGrn 3-UV



A) Current config

B) date of last CST run

C) pass/fail

D) Run CST

E) bead lot selection

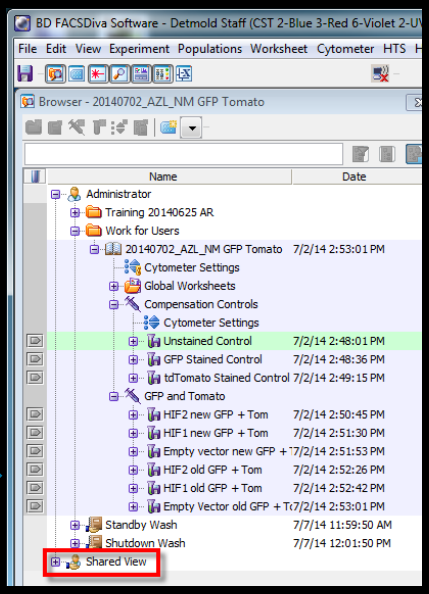
CST TROUBLESHOOTING

- **No events** can result from: ✹ blockage, ✹ cytometer not in RUN-mode, ✹ fine-adjustment knob is fully turned anti-clockwires (i.e, no flow), ✹ Lasers turned off, ✹ wrong 5mL tube used, ✹air-lock / machine run dry, ✹ Software glitch
- **System unable to identify beads** – bead concentration may not be appropriate – did you vortex the CST tube before making up the beads?
- **Pass with warnings:** View the report. The parameter with the error will be highlighted in red. If the error is the CV, the system will probably be OK to run, assuming you are not doing DNA analysis. Please tell a staff member if several CST runs have failed in a row.
- **Failed:** Check UV laser was turned on. Otherwise consult facility staff.

STANDY BY PROCEDURE

Every time you run samples on the Fortessa, the instrument needs to be washed at the completion of your session.

If someone is using the instrument after your booking, please do a standby wash.
If you are the last user of the day please refer to the **Fortessa Shutdown Procedure.**



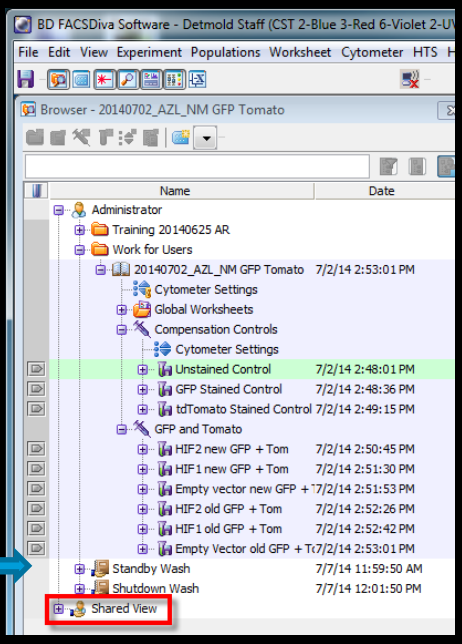
1. If next booking will be in > 30 min away, turn off Lasers
2. In the cytometer **Browser** window, scroll to the bottom and expand the **Shared View** section
3. **Double click** on the **Standby Wash** experiment to open the wash profile
4. Expand the Wash tubes specimen to access the wash tubes: Bleach, FACSclean and Water
5. Click on the acquisition marker beside the first tube in the list (Bleach)
6. Load a tube of bleach onto the cytometer, ensuring that the cytometer is set to RUN not Standby and on **HI flow rate**
7. On the computer, press **Record Data**, when asked, choose to Overwrite the existing data
    - The tube will run for 2 minutes
8. When the tube has finished running/recording, unload the tube, click **Next Tube** in the Acquisition Dashboard, then repeat steps 5-7 with both **FACSclean** and then with **Water**
9. Leave the water tube loaded on the cytometer and press **standby** on the front panel
10. Log out of the software and leave ready for the next user

<u>Changed configuration?</u> YOU WILL NEED TO RETURN THE ALTERNATE FILTERS/DICHROIC MIRRORS, SIGN THE FILTER REGISTER AND RETURN THE REMINDER NOTICE

SHUT DOWN PROCEDURE

Every time you run samples on the Fortessa, the instrument needs to be washed at the completion of your session.

If you are the last scheduled user for the day, please run a full shut down wash to ensure the ongoing operation of the machine.



1. Turn off Lasers.
2. In the cytometer **Browser** window, scroll to the bottom and expand the **Shared View** section
3. Double click on the **Shutdown Wash** experiment
4. Expand the Wash tubes specimen to access the wash tubes: Bleach, FACSclean and Water
5. Click on the acquisition marker beside the first tube in the list (Bleach)
6. Load a tube of bleach onto the cytometer, ensuring that the cytometer is set to RUN (not Standby) and on HI flow rate
7. On the computer, press **Record Data.** When asked, choose to Overwrite the existing data
    - The tube will run for 5 minutes
8. When the tube has finished running/recording, unload the tube, click **Next Tube** in the Acquisition Dashboard, then repeat steps 4-6 with both **FACSclean** and then with **Water**
9. Leave the water tube loaded on the cytometer and press standby on the front panel
10. Log out of FACSdiva software
11. Do not close BD Coherent Software (laser control).
12. Leave the computer logged into Windows
13. Turn off Fortessa (green button)

<u>Changed configuration?</u> YOU WILL NEED TO RETURN THE ALTERNATE FILTERS/DICHROIC MIRRORS, SIGN THE FILTER REGISTER AND RETURN THE REMINDER NOTICE

Changing FACS-flow sheath-carton

Once alarm sounds, and FACS flow empty light is on:

1. Finish recording data for current tube
2. Put O/N or water tube on the sample probe
3. Put Fortessa into stand by
4. Unscrew and remove empty sheath carton
5. Put empty carton to one side; slide full carton in place
6. Remove cap from full carton; put cap on the empty one
7. Insert sheath-line into full carton and screw in place. Do not over tighten. Pull the thread on the carton up, so the sheath-line fits in properly.
8. Press **[Reset Alarm]** on the fluidics cart and make sure the red light turns off

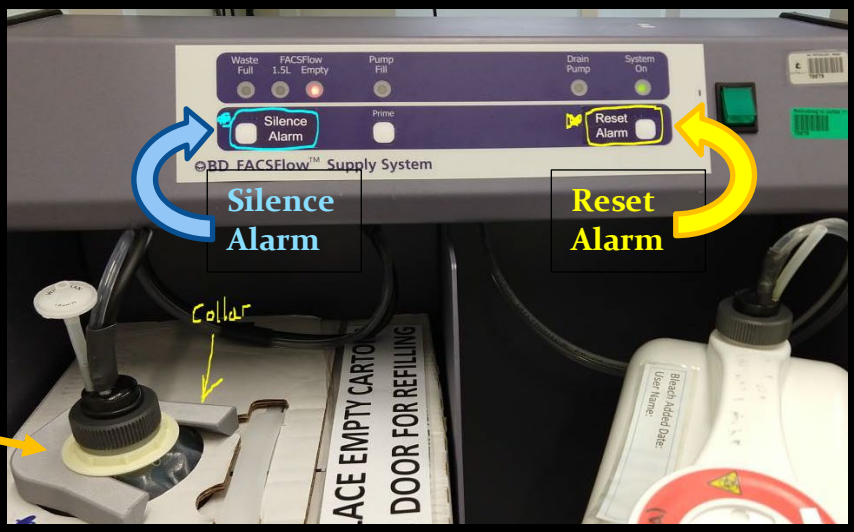
   Pressing [silence alarm] only silences the alarm – need to press [reset alarm]
9. Fortessa can be put in run-mode to resume your work

Changing waste tank

Once alarm sounds, and Waste full light is on:

1. Finish recording data for current tube
2. Put O/N or water tube on the sample probe
3. Put Fortessa into stand by
4. Unscrew waste line from full waste tank and set to one side
5. **Remove the rear-cap on the empty waste tank and fasten cap on full waste tank**
   a. *To avoid spills, install the rear cap on the full tank before removing it from the cart*
6. Remove full waste tank from fluidics cart and install empty tank.
7. Screw waste-line onto the empty waste tank. Do not over tighten
8. Press **[Reset Alarm]** on the fluidics cart and make sure the red light turns off

   Pressing [silence alarm] only silences the alarm – need to press [reset alarm]
9. Decontaminate the full waste tank: add 1 litre of sodium hypochlorite to waste tank, write date on waste container and place on the wheeled trolley (PC2 requirement).
10. Fortessa can be put in run-mode to resume your work



Collar to assist in reattaching fluidics line

Blockage / no events

If CST errors and no events were observed in the first (of five) plots:

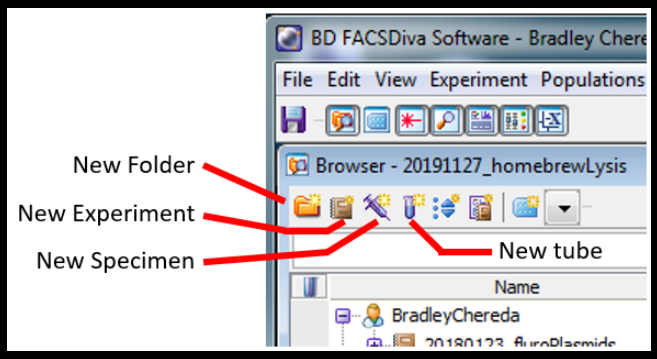
1. Check the fine adjustment dial is not fully turned anti-clockwires.
2. Check cytometer in [Run] mode
3. Check that Lasers are on
4. Check sheath carton is not empty
5. Check that FACS-flow (sheath) red light is not lit on the fluidics cart – if it is, get a staff member.
6. Bleed air from sheath filter
7. Remove CST tube and set-aside. Place bleach tube on sample probe and put cytometer on [HI].
8. Exit CST
9. (optional) Log out of user name and log into CST_wash (pw: muse42)
10. Open an experiment and make an FSC vs SSC dot plot
11. Acquire (not record) data
12. Wait for events to be on screen, or 3-5 minutes.
13. a/ If events observed: run water tube for 2 minutes, then resume CST (put flow rate back to [low].
    b/ If no events:
    - restart machine – waiting 2 minutes before turning Fortessa back on.
    - find/contact staff member – 8302 7809
    - continue bleach tube for 10-15 minutes.

If during run:

1. Check the fine adjustment dial is not fully turned anti-clockwires.
2. Check cytometer in [Run] mode
3. Check that Lasers are on
4. Check sheath carton is not empty
5. Check that FACS-flow (sheath) red light is not lit on the fluidics cart – if it is, get a staff member.
6. Bleed air from sheath filter
7. (optional) Log out of username and log into CST_wash (pw: muse42)
8. Open an experiment and make an FSC vs SSC dot plot
9. Place bleach tube on sample probe and put cytometer on [HI] flow rate.
10. Acquire (not record) data
11. Wait for events to be on screen, or 3-5 minutes.
12. a/ If events observed: run water tube for 2 minutes, then resume run.
    b/ If no events:
    - restart machine – waiting 2 minutes before turning Fortessa back on.
    - find/contact staff member – 8302 7809
    - continue bleach tube for 10-15 minutes.

Running a New Experiment

Experiment setup



- To create a new experiment. In the **Browser** window click the [New Experiment] icon

- To duplicate a previous experiment
  - Double-click and open old experiment
  - Right click and select [Duplicate Without Data]

- Rename the experiment to fit the required convention **20191204_BC_Exp name**

  That is: (year month day) YYYYMMDD_your initials_experiment name.

  It **must** be in this format for proper data management.
  - The data will be saved in a folder with the name of your experiment. This folder is created when you record data for your first tube. Changing the experiment name later will create another folder with subsequent data saved within.

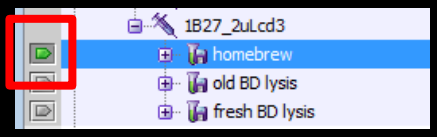
Tube setup

For new experiments (not duplicated), create one specimen/tube, setup the parameters and set the appropriate voltage values from a full stained tube. Then create compensation and sample tubes. If you make all the tubes first, sometimes voltages will not be applied across the tubes correctly.

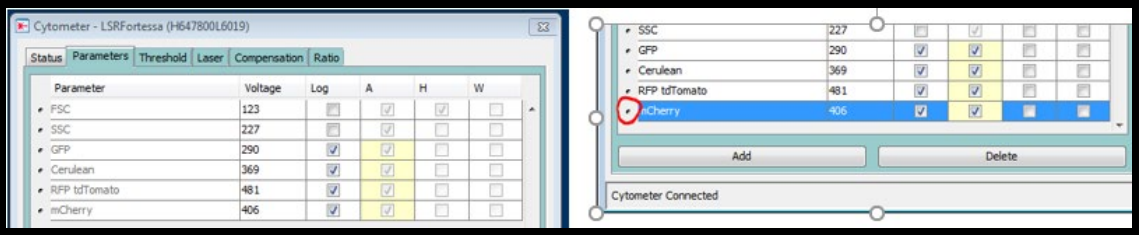
- Create a specimen with the [add specimen] icon.
- Expand specimen by pressing the plus sign next to the Syringe icon, you should now see one tube in your list
  - Once a tube has finished recording, it is immediately exported. Any sample renaming in Diva after recording will not be reflected in the filename. You will have to manually export the FCS file again.

Parameter setup

- Click on the **Acquisition Marker** beside any tube
  - *Clicking on the tube name is not sufficient. You need to click the **Acquisition Marker**, it will become active (green).*



- The cytometer controls will activate. In the cytometer window, you can then make changes to the parameters: add/delete, change voltage, compensation, threshold etc.



  - Setup parameter names. Find the fluorochrome in the channel listing. For example, clicking on Alexa Fluor 488 – to bring up the dropdown list, and selecting GFP. If your fluorophore isn't listed, use the generic name. For example: (V 525/50) V for violet, 525/50, the bandpass of the detector. You can add a custom label using the [experiment layout] – see below.
  - Delete unwanted parameters. This will reduce file size and make it easier to setup the cytometer/layout. Click on the small dot next to the parameter(s) you wish to delete and then press

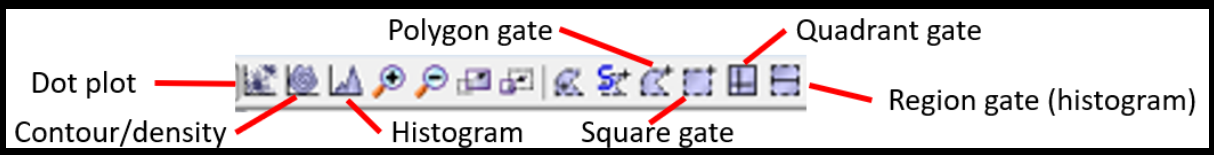
the Delete button at the bottom right. *Do this after setting up your parameter names, otherwise when you click on a parameter name, it will list the names of that detector AND all of the deleted ones.*

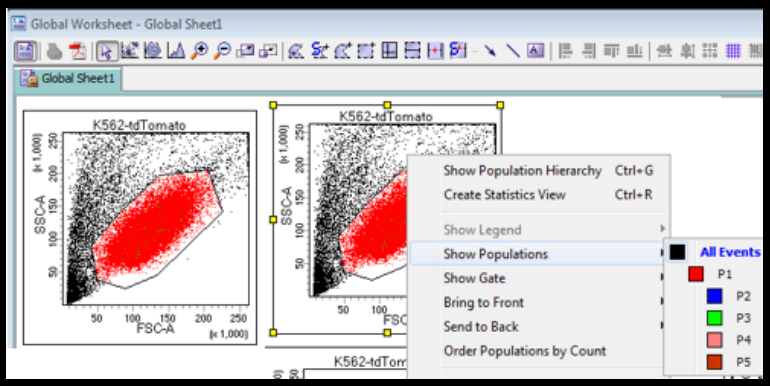
- Ensure H, A, W parameters are selected as needed (e.g. "H" and "A" for FSC to enable doublet discrimination).
- The Log column determines Log/Linear settings. The defaults are, FSC and SSC: linear [unchecked]; fluorescence: Log [checked]

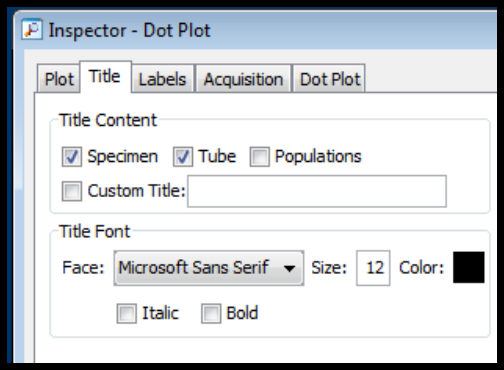
- Use the **Experiment Layout** window to edit axes labels, acquisition number and duration and stopping gate.

  From the **Experiment** menu at the top of the window, scroll down and click on **Experiment Layout**

Create plots







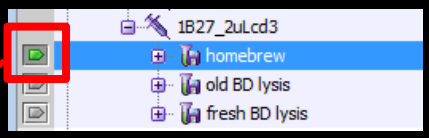
- On the **Worksheet** (right screen), you can create plots and setup gates using the buttons across the top of the Worksheet window.

<u>Suggested basic layout for new assays</u>:

- Create two dot plots: FSC-A/SSC-A and FSC-A/FSC-H.
- To help set voltages, make bi-exponential histograms for each fluorophore – put them in the grey region so they aren't included in your PDF (bi-exp option: click on plot; inspector window and [plot] tab).
- You can then set up the plots you want to print out within the white area.

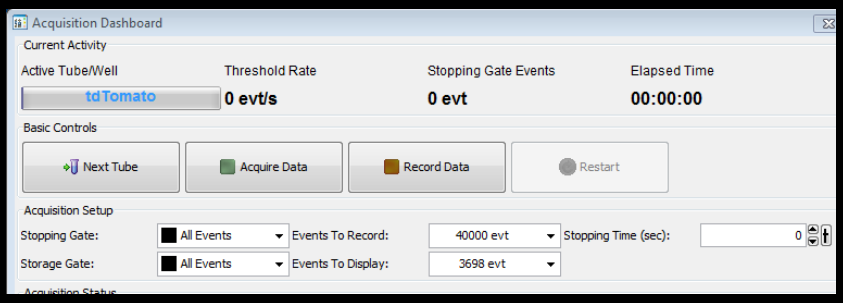
- To display events from inside a specific gate on a certain plot, right click on the plot, click **Show Populations**, and select the population(s) you wish to view.
- To show the gating hierarchy, press ctrl + G.
- For custom plot titles, click on all the plot(s). In the [Inspector] window, select the [**Title**] tab. Select from [specimen], [tube], [population] *the population shown in that particular plot*, or create a custom title.

Acquiring data workflow



New users: read entire section before proceeding

1) Select your first tube by clicking on its **Acquisition Marker**
2) Check [events to record] value is appropriate (acquisition dashboard)
3) Press **Run** on the front panel of the cytometer, have flow rate on [Lo]
4) Move the sample arm to the side
5) Load your sample tube and move the sample arm back under the tube without delay (cytometer will quickly aspirate sample when the arm is to the side).



6) Click [Acquire data] on the **Acquisition Dashboard** and data will appear within your plots.
7) Set parameter voltages (or check the previous voltages are appropriate).
8) Click [record] data to record/save data

   Once the tube has been "recorded", the data will be saved and exported. You may then load your next tube. Ensure that the **Acquisition Marker** is set to the appropriate tube by either clicking on it manually, or, if you are remaining within one **Specimen**, clicking on **Next Tube** in the **Acquisition Dashboard**. then press **Acquire**, and **Record** when you are ready.

Acquiring data for a new experiment

- Follow 1-7 of acquiring data workflow, loading a full stained control tube (for voltage setting)

7) Set parameter voltages

The **voltage parameter** is used to change the signal intensity (like the volume control on a radio). If you want the signal to increase; increase the voltage; decrease the voltage to reduce the signal. The default voltages are derived from CS/T, based on the best fluorescence separation of neg, dim, bright bead-populations. Try to keep within 50 volts of the defaults. Titrating the antibody/stain to fit in the CS/T voltages will get better results compared to altering the voltages to fit an arbitrary stain volume.

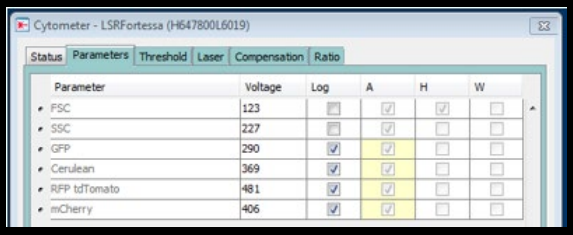
Voltages CAN NOT be changed between tubes (except for FSC and SSC). So make sure you use a suitable full stain control to set them correctly.

Voltages can be changed in the cytometer window.

- select/highlight number and type a new number

- select/highlight number press the up or down arrow on the keyboard. Ctrl + arrow changes by 10 volts

- Ballpark for FSC: 123 volts; SSC 222 volts



8) click [record] data to record/save data.

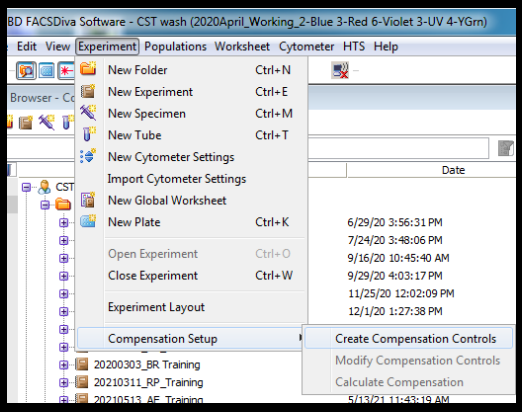
9) create appropriate specimens and tubes for the rest of your samples. See next section for auto-compensation setup.

COMPENSATION – calculating and modifying

Compensation settings can be adjusted at anytime.
So long as the instrument voltages are set correctly, you can edit the compensation values during data analysis.

Auto-Compensation

- Check that only the parameters you are measuring are listed in the parameter list
- Click [experiment] in the top menu bar, mouse over [compensation setup] and click [create compensation tubes].

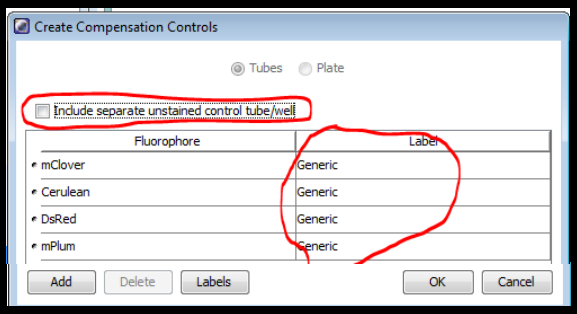


Parameter setup

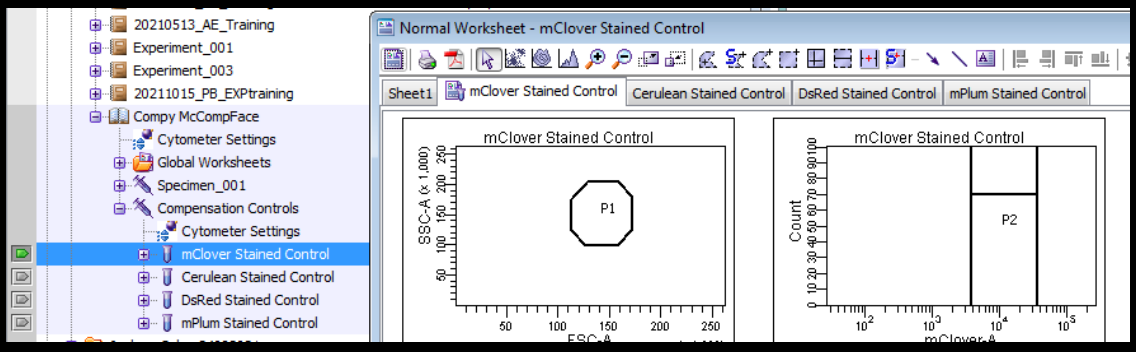
- Include separate unstained tube:

  Select if using an unstained tube control

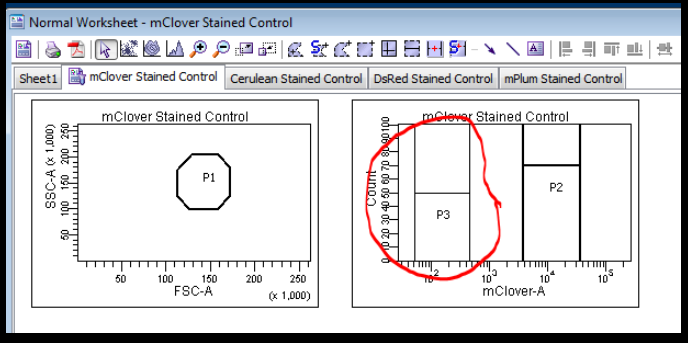
  Deselect if using the negative population within your single stain controls.
- Delete fluorophores you don't have controls for
- Delete fluorophores with custom labels (ie, only have fluorophores with a "generic" label).



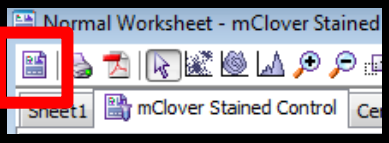
- FACSDiva will generate a specimen and tubes for your single stained controls as well as a global worksheet for gating the data on each tube.



- If you deselected separate unstained tube, you need to add a negative population interval gate for each tube.



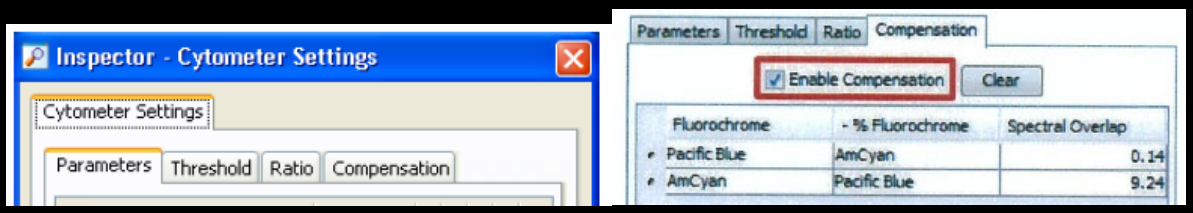
- Load your single stain tubes and record data for each
- Apply appropriate gates for each control.
- Click [experiment] in the top menu bar, mouse over [compensation setup] and click [calculate compensation].
- Diva may alert you there are large compensation values in the experiment. This does not necessarily mean that auto-comp will be unsuitable for your experiment, but you should double-check to make sure that the values are correct.
- When asked by the system, add your initial to the end of the date and time stamp name that has already been generated, and make sure that you press **Link and Save**, do not select [apply].
- The compensation and voltages will be applied to new specimens and those with no data.
- Make sure to change back to "global worksheet" before running sample tubes in order to see your plots/data.



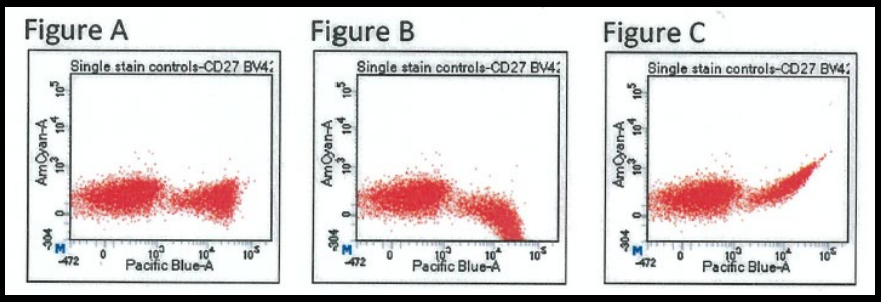
Manual compensation

Changing a compensation value can affect multiple parameters. This section explains which fluorochrome to subtract from a detector. The example contains just 2 fluorochromes (Pacific Blue and AmCyan), but the principles remain the same regardless of the number of fluorochromes you are analysing.

- Record data for all of the single stained compensation controls.
- Select the tube you wish to view (by clicking the **Acquisition Marker**).
- In the **Inspector** dialog and **Compensation** tab, ensure that **Enable compensation** is selected. This selector allows you to turn compensation on and off (on a tube-by-tube basis)



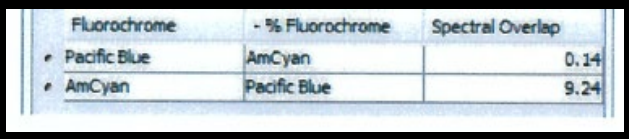
- Create a matrix of plots (i.e. plots showing every fluorochrome plotted against every other fluorochrome)
- Highlight all these plots, and in the **Inspector** on the **Plot** tab, ensure that in the Biexponential Display section, both **X Axis** and **Y Axis** are selected
    - Biexponential display allows for pseudo negative numbers in a log scale. So, you can visualise all the data points if they go off-scale due to spectral overlap or applied compensation.



A) properly-compensated data *B) over-compensated data* *C) under-compensated data*

- The above example is a Pacific Blue single stain control. Pacific Blue *should not* be seen as AmCyan positive, and needs to be compensated out. Therefore, we are looking to subtract Pacific Blue fluorochrome from the AmCyan detector.
    - In the compensation window, the columns are delineated as:

Fluorochrome | - % Fluorochrome | Spectral Overlap



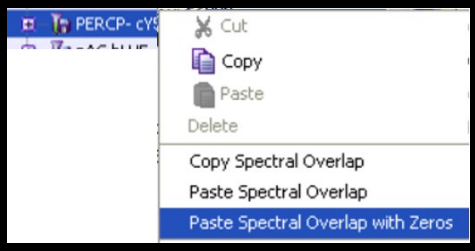
Fluorochrome	- % Fluorochrome	Spectral Overlap
Pacific Blue	AmCyan	0.14
AmCyan	Pacific Blue	9.24

    - To subtract Pacific Blue from AmCyan:

      Fluorochrome = AmCyan

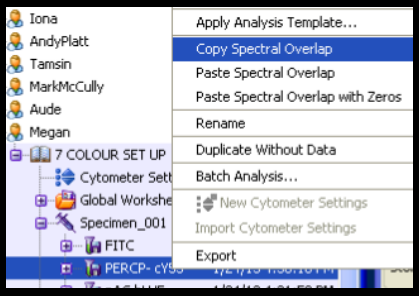
      - % fluorochrome = Pacific Blue
    - When you have AmCyan in the tube and are looking to subtract it from the Pacific Blue detector, the equation is reversed and becomes **Pacific Blue - % AmCyan**

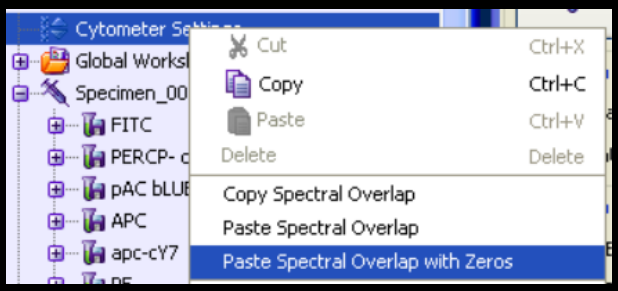
- After setting the compensation for the first tube, you will need to copy that altered compensation setup (**Copy Spectral Overlap**) and paste it onto the subsequent tube (**Paste Spectral Overlap with Zeros**). These options are found by right-clicking the tube.



- Check the compensation for the next tube(s), and copy and paste the spectral overlap as necessary. When you have iterated through all the tubes, copy the final tubes spectral overlap onto the other tubes, so they all have a complete matrix.

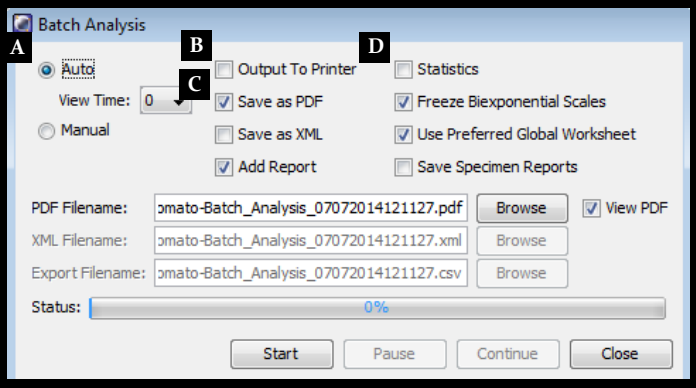
- Copy the "complete matrix (spectral overlap)" and paste onto the experiment **Cytometer Settings**. This will apply the overlap on all future tubes.
    - If you have run Auto-comp you will first need to unlink the Experiment **Cytometer Settings** (the settings with the gold chain link icon), by right clicking on the **Cytometer Settings**, and selecting **Unlink from 20190512103258 BC*** (this name will be different and based on a date and time stamp and your own initials)





BATCH ANALYSIS – Printing, PDF reports and Statistic Exports

- To print the Worksheet, create a PDF of the results, or export statistics for all your samples, highlight all the specimens you require information from (or select individual tubes if not all are required)
- Select specimen or tubes
    - **Specimen:** hold [ctrl] and click the specimen(s) for export
    - **Tubes**: hold [ctrl] and click the tubes for export (can not select tubes over different specimens)
- Right click on a selected specimen/tube select **Batch Analysis**



- A - If no gate changes are required from sample to sample, change **View Time** to **0** to make the process as fast as possible
- A - If you have different cell types and need to adjust gates between sample types, switch to **Manual** mode so that you can make changes before proceeding with the export/print between tube files
- B – [deselect] - there is no printer attached to the fortessa PC

- C – [select] **make sure the PDF filename saves to your <u>own folder</u>**
    - Folder will be in **D:\BDExport\Worksheet\Your Name**
    - this file will be available on the network the following day as per standard data backup policy
    - for genuine urgent data requests please make arrangements with staff *prior* to your acquisition run
- D – if you wish to export statistics, check this box, **make sure the output will go to your <u>own folder</u>**
    - Folder will be in **D:\BDExport\Statistics\Your Name**
    - this file will be available on the network the following day as per standard data backup policy
    - for genuine urgent data requests please make arrangements with staff *prior* to your acquisition run

GETTING FLOW DATA

Each morning (9 am), the FCS data from the previous day will be uploaded to the cloud. The backup can only occur of the cytometer-PC is logged into Windows.

CLOUD ACCESS

2-factor authentication

Research data storage now requires 2-FA when logging in.
I recommend to use the "google authenticator" app (android/iOS) instead of the one uniSA recommends as there has been issues with it.

Users with a university email

You will need to log into the RDS (research data storage) system (you don't have to apply for storage) **https://rds.unisa.edu.au**
You will not see any folders on first log in.
Once you log in, let a staff member know and they can share the flow data folder with you.

External users (without a uniSA email address):

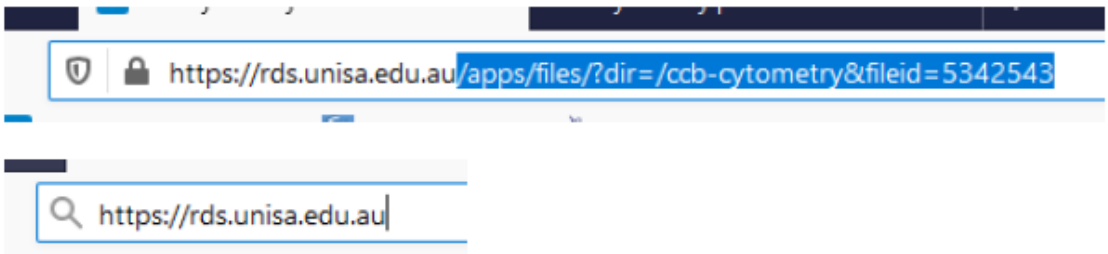
ITS has enabled onerous authentication requirements for non @unisa.edu.au accounts please discuss access options with staff (your data will be backed up on the server).

File Paths

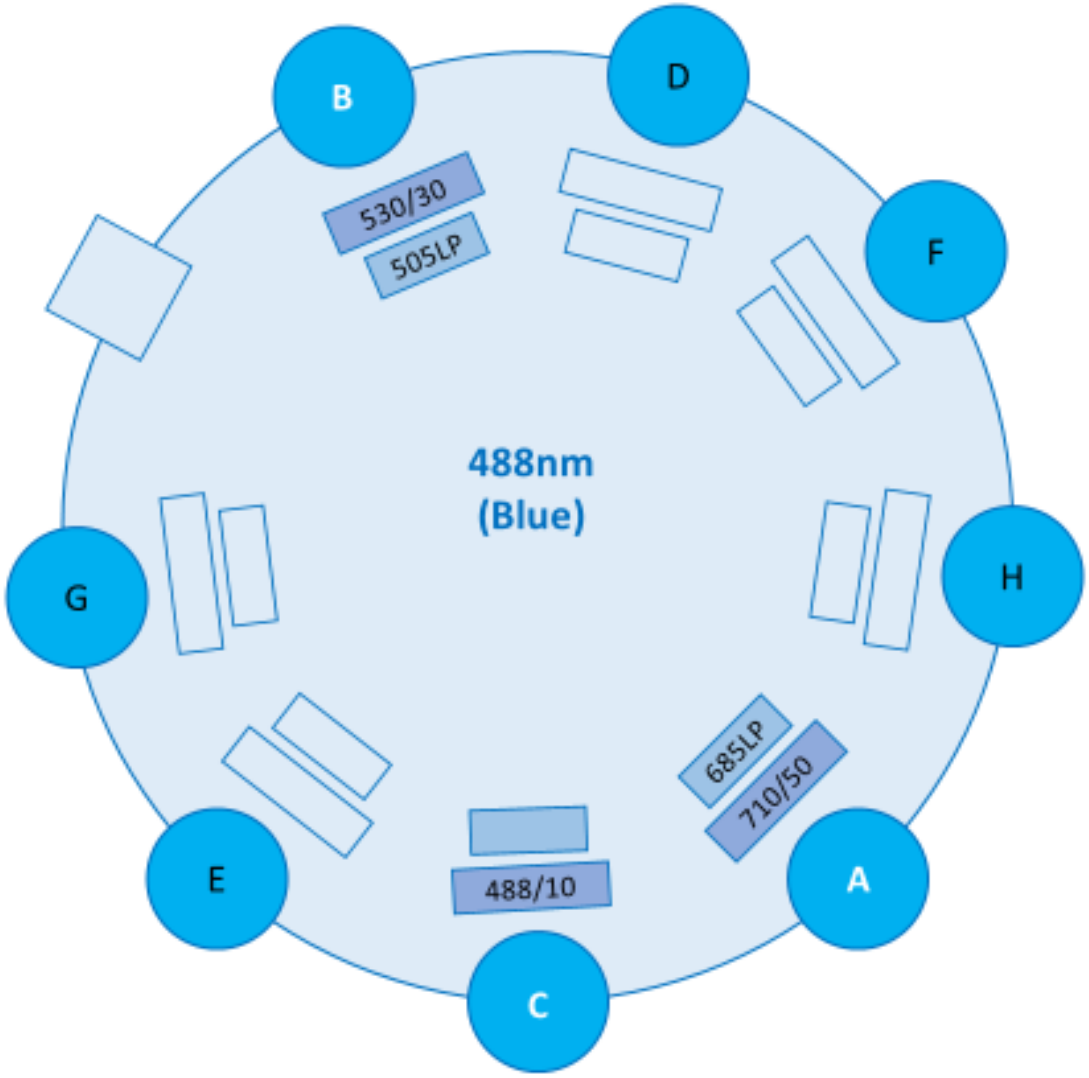
Fortessa	FCS	\fortessa\data\FCS\yourusername
	PDF	\fortessa\data\Worksheet\yourusername
Gallios	LMD	\Gallios\data\yourusername\LMD
	PDF	\Gallios\data\yourusername\PDF
Astrios (sorter)	FCS / screencaps	\Astrios\data\Institute\LabName\LMD\year\Username\date

CLOUD BUG

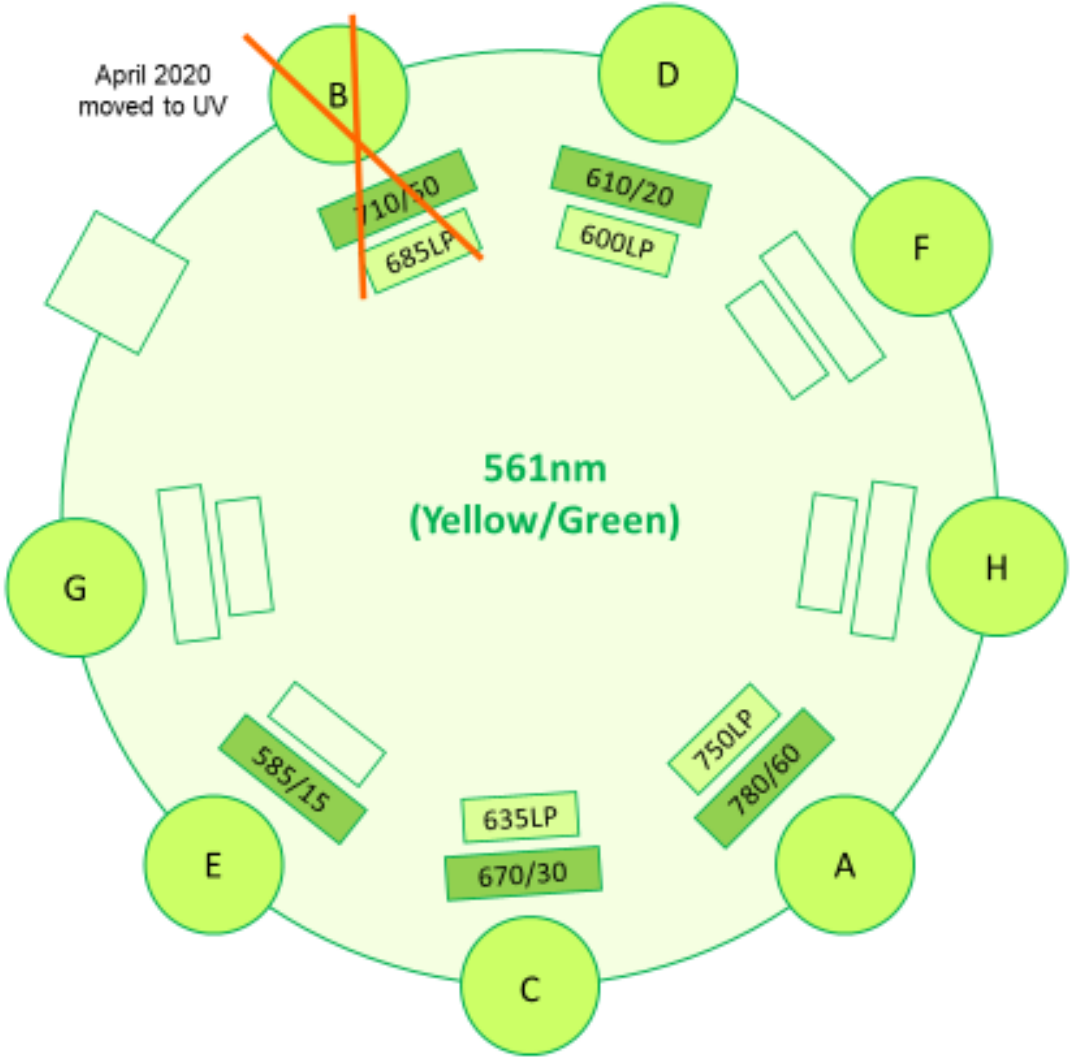
Upon logging in to RDS, your browser may not display anything. In the address bar delete the highlighted text (so that only "https://rds.unisa.edu.au" remains)



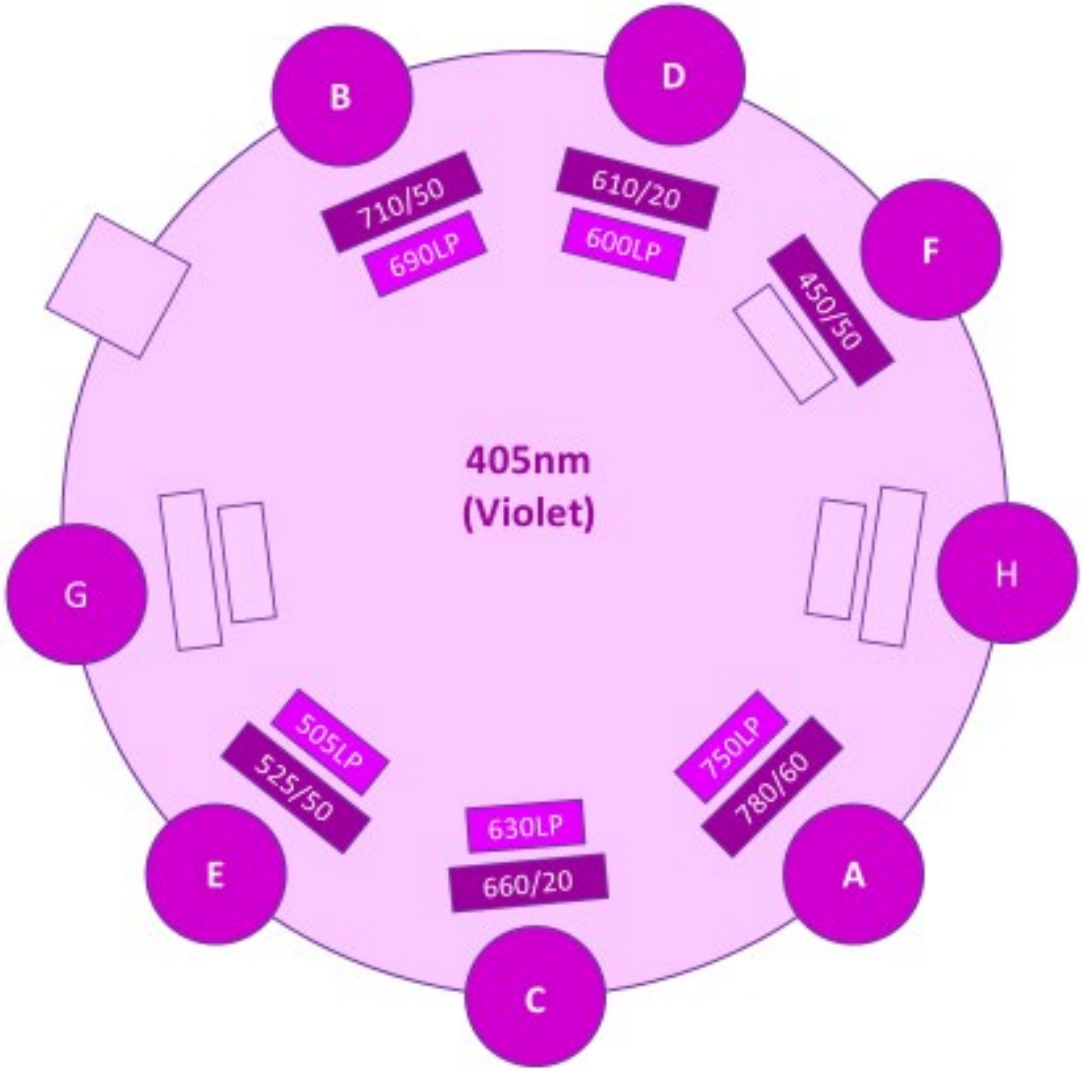
And press enter; you should now see the normal interface.



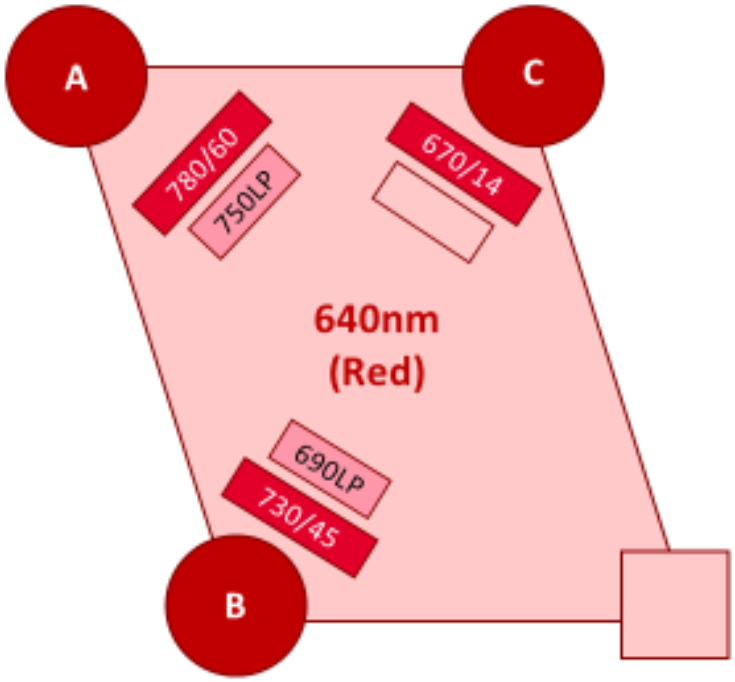
Detector A	B 710/50
7-AAD	BB700
PerCP	B 710/50
PerCP-Cy5.5	FVD eFluor 520
PE-Cy5.5	PerCP-eFluor 710
PI	PerCP-Vio700
Detector B	**B 530/30**
Alexa Fluor 488	Cy2
FITC	DyLight488
GFP	FVS 520
BB515	Live/Dead Green
B 530/30	Oregon Green
Calcein-AM	mClover
CFSE	Venus
	YFP
Detector C	**488/10***
488nm SSC	This auto-selects, and no other parameter can be recorded in this channel



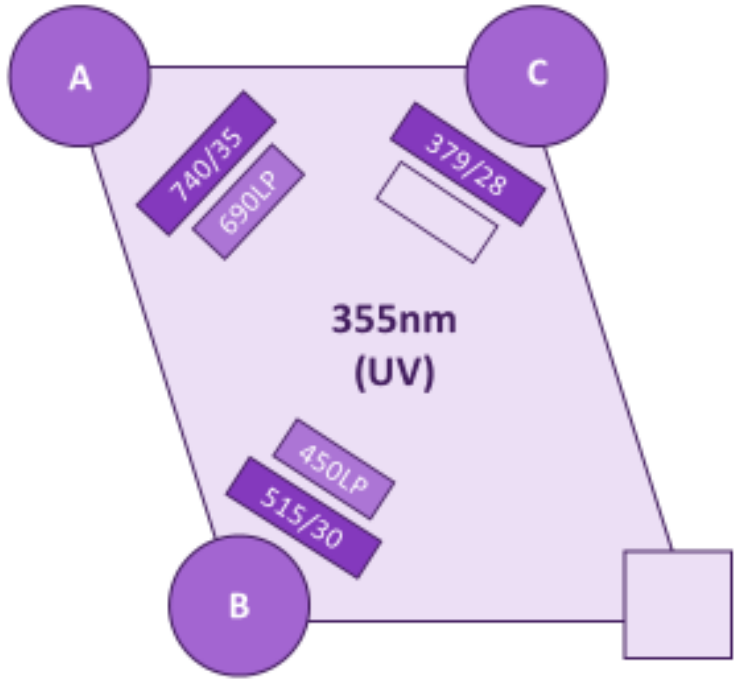
Detector A	Y 780/60
PE-Cy7	Y 780/60
PE-Vio770	
~~Detector B~~	~~Y 710/50~~
~~PE-Cy5.5~~	Y 710/50
Detector C	**Y 670/30**
PE-Cy5	Y 670/14
7-AAD (561ex)	Y 670/30
mPlum	
Detector D	**Y 610/20**
PE-CF594	mCherry
PE-Texas Red	Nile Red
Alexa Fluor 568	PI (561ex)
ECD	Y 610/20
Detector E	**Y 585/15**
DsRed	mRuby
PE	RFP
Alexa Fluor 546	SNARF
Alexa Fluor 555	tdTomato
Cy3	Y 585/42
eFluor 585 NC	Y 586/15



Detector A	V 780/60
BV786	V 780/60
Detector B	**V 710/50**
BV711 Qdot 700	V 710/50
Detector C	**V 660/20**
BV650	V 660/20
Detector D	**V 610/20**
BV605 Qdot 605	V 610/20
Detector E	**V 525/50**
Alexa Fluor 430 AmCyan BV480 BV510 FVS 510	Pacific Orange Krome Orange V500 V 525/50
Detector F	**V 450/50**
Alexa Fluor 405 BV421 Cascade Blue CFP Pacific Blue V450	Cerulean DyLight 405 FVD eFluor 450 FVS 450 V 450/50



Detector A	R 780/60	
APC-Cy7 & APC-H7	APC-Alexa750	FVS 780
APC-Vio770	APC-eFluor780	R 780/60
Detector B	**R 730/45**	
Alexa Fluor680	FVS700	R730/45
Alexa Fluor700	APC-R700	
Detector C	**R 670/14**	
Alexa Fluor633	APC	Live/Dead Far Red
Alexa Fluor647	Cy5	R 670/14
eFluor 660	DRAQ5	R 670/30
FVD eFluor 660	FVS 660	TO-PRO-3



Detector A	UV 740/35
Hoechst Blue	Qdot 800
BUV 737	UV 740/35
Detector B	**UV 515/30**
BUV 496	UV 585/42 (need to change filter)
UV 515/30	UV 675/50 (need to change filter)
Detector C	**UV 379/28**
Alexa Fluor 350	DAPI, Hoechst 33342 (450 nm
BUV 395	dyes) ask staff for filter change

Fluorochrome	Excitation (nm)	Emission (nm)	Best Filter (available)	LP	Alternative Filters (all non-standard)	Notes
7-AAD	488;546	647	710/50;670/30	685;635	670/30 BP with 635LP*	NOTE: very high spectral spillover into 670/30 BP from 561nm Yellow laser
Alexa Fluor 350	343	442	450/50	-		
Alexa Fluor 405	401	421	450/50	-		
Alexa Fluor 430	434	540	525/50	505		
Alexa Fluor 488	499	519	530/30	505		
Alexa Fluor 500	503	525	530/30	505		
Alexa Fluor 514	517	542	530/30	505		
Alexa Fluor 532	530	555	530/30	505	560/40 BP with 505LP	
Alexa Fluor 546	561	572	586/15	-	575/25 BP with 550LP	Alternative filter set should only be used if using 488nm Blue laser for excitation
Alexa Fluor 555	553	568	586/15	-	575/25 BP with 550LP	Alternative filter set should only be used if using 488nm Blue laser for excitation
Alexa Fluor 568	579	603	610/20	600		
Alexa Fluor 594	591	618	610/20	600		
Alexa Fluor 610	610	629	610/20	600		
Alexa Fluor 633	632	648	670/14	690	670/30	
Alexa Fluor 647	652	668	670/14	-	670/30	
Alexa Fluor 660	663	691	675/50*	-		This filter combo is **NON**-standard for 640nm Red laser
Alexa Fluor 680	680	702	730/45	690		
Alexa Fluor 700	696	719	730/45	690		
Alexa Fluor 750	752	804	780/60	750		Currently unable to excite this dye with available lasers
Alexa Fluor 790	782	804	780/60	750		Currently unable to excite this dye with available lasers
AmCyan	458	489	525/50	505		
APC	645	660	670/14	-	670/30	
APC-Alexa 680	650	702	730/45	690		
APC-Alexa 700	650	719	730/45	690		
APC-Alexa 750	650	774	780/60	750		
APC-Cy5.5	650	690	730/45	690		
APC-Cy7	650	774	780/60	750		
APC-eFluor 780	650	780	780/60	750		
APC-Fire 750	650	787	780/60	750		
APC-H7	650	785	780/60	750		
APC-R700	652	704	730/45	690		
APC-Vio770	652	775	780/60	750		
Azami Green	492	505	530/30	505		
Azurite	355;405	450	450/50*	535	(UV) 450/50	UVB: 535LP and 560/20 UVC: 450/50
BB515	490	515	530/30	505		
BB630	484	631	Not released	NR		
BB660	484	667	Not released	NR		
BB700	484	695	Not released	NR		
BB790	484	793	Not released	NR		
BFP	380	440	450/50	-		
BUV395	348	395	379/28	-		
BUV496	348	496	525/50*	505		This filter combo is **NON**-standard for 355nm UV laser
BUV563	348	563	585/15*			This filter combo is **NON**-standard for 355nm UV laser
BUV615P	349	616	Not released			

BUV661	348	661	660/20*	630		This filter combo is **NON**-standard for 355nm UV laser
BUV737	348	737	740/35	685		
BUV805	348	805	780/60*	750		This filter combo is **NON**-standard for 355nm UV laser
BV421	407	421	450/50	-		
BV480	436	478	525/40*			This filter combo is **NON**-standard for 405nm Violet laser
BV510	405	510	525/50	505		
BV570	407	574	Not released			
BV605	407	605	610/20	600		
BV650	407	650	660/20	630		
BV711	407	711	710/50	690		
BV750P	407	748	Not released			
BV786	407	786	780/60	750		
BYG584	563	584	Not released			
Calcein Blue-AM	360	445	450/50	-		
Calcein Violet-AM	408	450	450/50	-		
Calcein-AM	495	515	530/30	505		
Cascade Blue	398	420	450/50	-		
Cascade Yellow	402	545	525/50	505		
Cell Proliferation Dye eFluor 450	409	450	450/50	-		
Cell Proliferation Dye eFluor 670	647	670	670/14	-	670/30	
CellTrace BODIPY	508	625	610/20*	600*		This filter combo is **NON**-standard for 488nm Blue laser
CellTrace Far Red	647	657	670/14	-	670/30	
CellTrace Oregon Green 488	498	516	530/30	505		
CellTrace Violet	405	455	450/50	-		
CellTracker Blue	353;371	466/464	450/50	-		CMAC/CMF_2HC
CellTracker Deep Red	630	650	670/14	-		
CellTracker Green	492	517	530/30	505		
CellTracker Orange	548	576	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
CellTracker Red	577	602	610/20	600		
CellTracker Violet	415	516	525/50	505		
CellVue Burgundy	683	707	730/45	690		
CellVue Claret	655	677	670/14	-		
CellVue Jade	478	508	530/30	505		
CellVue Lavender	420	461	450/50	-		
CellVue Maroon	647	667	670/14	-	670/30	
CellVue NIR780	745	776	780/60	750		Currently unable to excite this dye with available lasers
CellVue NIR815	786	814	780/60	750		Currently unable to excite this dye with available lasers
CellVue Plum	652	671	670/14	-		
Cerulean	433	475	450/50	-		
CFP	433	475	450/50	-		
CFSE	494	521	530/30	505		
Chromeo 488	488	517	530/30	505		excellent dye for STED microscopy
Chromeo 494	494	628	610/20*	600*		This filter combo is **NON**-standard for 488nm Blue laser, excellent dye for STED microscopy
Chromeo 505	505	526	530/30	505		excellent dye for STED microscopy
Chromeo 546	545	561	585/42*	-	586/15	This filter combo is **NON**-standard for 561nm Yellow/Green laser, excellent dye for STED microscopy
Chrome 642	642	660	670/14		660/20	excellent dye for STED microscopy
Chromomycin A3	445	575	450/50			we do not have the optimal laser for exciting this fluorochrome is wishing to analyse/sort chromosomes
Cy2	489	506	530/30	505		
Cy3	550	570	586/15	-	575/25 BP with 550LP	Alternative filter set only to be used if using 488nm Blue laser for excitation, filter set is **NON**-standard for 488nm Blue laser

Cy3.5	581	594	586/15	-		
Cy5	650	670	670/14	-	670/30	
Cy7	743	767	780/60	750		
CyChrome	480	670	710/50	685	670/30 BP with 635LP	
CytoPhase Violet	355	440	450/50	-		
DAPI	345	455	450/50	-		
DiI	549	565	530/30	505	560/40 BP with 505LP	
DiO	484	501	530/30	505		
DRAQ5	647	681,697	670/14	-	670/30	
DRAQ7	600;646	697	685/35	-	670/30 BP or 675/50 BP	WARNING: multilaser excitation - 561 and 488nm lasers will also excite DRAQ7
DsRed	558	583	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
dTomato	554	581	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
DyLight 350	353	432	450/50	-		
DyLight 405	400	420	450/50	-		
DyLight 488	493	518	530/30	505		
DyLight 550	562	576	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
DyLight 594	593	618	610/20	600		
DyLight 633	638	658	670/14	-	670/30	
DyLight 650	654	673	670/14	-		
DyLight 680	692	712	730/45	690		
DyLight 755	754	776	780/60	750		Currently unable to excite this dye with available lasers
DyLight 800	777	794	780/60	750		Currently unable to excite this dye with available lasers
ECD	486	620	610/20*	600*		This filter combo is **NON**-standard for 488nm Blue laser
eFlour 565 NC	550	565	530/30	505	560/40 BP with 505LP	
eFluor 490 NC	470	490	530/30	505		
eFluor 525 NC	505	525	530/30	505		
eFluor 585 NC	570	585	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
eFluor 605 NC	590	605	610/20	600		
eFluor 625 NC	610	625	610/20	600		
eFluor 650 NC	640	650	670/14	-		
eFluor 660	633	660	670/14	-	670/30	
eFluor 700 NC	660	690	730/45	690		
Emerald	487	509	530/30	505		
FITC	495	519	530/30	505		
Fluo-3	506	526	530/30	505		
FluoroGold	330,390	450,600	450/50;585/42*	-		585/42 BP filter is **NON**-standard. **NOTE: will spill into 450/50 BP and 586/14 BP from 405nm Violet laser**
FluorX	494	520	530/30	505		
FP	-	-				
Fura-2	300-400*	510	530/30;525/50	505		Variable excitation based on concentration of free Ca^{2+}. 530/30 with UV, 525/50 with 405nm
FVD eFluor 450	405	450	450/50	-		
FVD eFluor 455UV	350	455	450/50	-		
FVD eFluor 506	405	506	525/50	505		
FVD eFluor 520	488	522	530/30	505		
FVD eFluor 660	633	660	670/14	-	670/30	
FVD eFluor 780	633	780	780/60	750		
FVS 450	406	448	450/50			
FVS 510	408	512	525/50	505		
FVS 520	498	521	530/30	505		
FVS 570	547	573	586/15	-	585/42	

FVS 620	523	617	610/20	600		
FVS 660	649	660	670/14	-	660/20	
FVS 700	657	700				
FVS 780	759	780				
GFP	488	507	530/30	505		
Helix NP Green	495	640	660/20*	600		This filter combo is **NON**-standard for 488nm Blue laser
Helix NP NIR	640	660	660/20*	-		This filter combo is **NON**-standard for 640nm Red laser
Hoechst	-	-	-	-		
Hoechst 33258	345	478	450/50	-		
Hoechst 33342	343	483	450/50	-		
Hoechst Blue	352	483	450/50	-		
Hoechst Red	352	650	670/30*	510*		This filter combo is **NON**-standard, but is best for Side Population analysis
Indo-1 (Blue)	350	475	450/50	-		
Indo-1 (Violet)	330	405	450/50	-		
Krome Orange	398	528	525/50	505		
Live/Dead Aqua	367	526	530/30	505		
Live/Dead Blue	350	450	450/50	-		
Live/Dead Far Red	650	665	670/14	-	670/30	
Live/Dead Green	495	520	530/30	505		
Live/Dead Near-IR	750	775	780/60	750		Currently unable to excite this dye with available lasers
Live/Dead Red	595	615	610/20	600		
Live/Dead Violet	416	451	450/50	-		
Live/Dead Yellow	400	575	525/50	505	610/20 BP with 595LP	
Lucifer Yellow	428	536	525/50	505		
Marina Blue	362	459	450/50	-		
mBanana	540	553	530/30	505	560/40 BP with 505LP	
mCherry	587	610	610/20	600		
mHoneyDew	487	537	530/30	505		
MitoTracker Deep Red	644	665	670/14	-	670/30	
MitoTracker Green	490	516	530/30	505		
MitoTracker Orange	551	576	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
MitoTracker Red	578	599	586/15	-		
mClover3	488	515	530/30	-		
mCyRFP1	488	585	586/15	-	586/15	Stand alone: detector B; swap 530/30 for 585/16. With GFP: detector A; install 586/15 with 545LP
mKate	588	633	610/20	600		
mNeptune	600	650	670/30	635		
mOrange	546	562	530/30	505	560/40 BP with 505LP	
mPlum	589	649	670/30	635		
mRaspberry	597	624	610/20	600		
mRFP1	584	607	610/20	600		
mRuby3	561	595	586/15	-		
mStrawberry	574	596	586/15	-		
mT-Sapphire	405	510	525/50	-		
mTangerine	568	585	586/16*	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
Nile Red	561	580	610/20			
Oregon Green 488	496	516	530/30	505		
Pacific Blue	410	455	450/50	-		
Pacific Orange	410	551	525/50	505		
PE	496;565	575	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP

PE-Alexa 594	496;565	618	610/20*	600*		
PE-Alexa 610	496;565	629	610/20*	600*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PE-Alexa 700	496;565	719	710/50	685		
PE-CF594	496;565	612	610/20*	600*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PE-Cy5	496;565	670	670/30*	635*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PE-Cy5.5	496;565	690	710/50	685	685/35 BP with 670LP	
PE-Cy5-5	496;565	690	710/50	685	685/35 BP with 670LP	
PE-Cy7	496;565	774	780/60*	750*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PE-Dazzle 594	566	610	610/20	600		
PE-mCherry	496;565	610	610/20*	600*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PerCP	482	675	710/50	685	670/30 BP with 635LP	
PerCP-Cy5.5	482	690	710/50	685	685/35 BP with 670LP	
PerCP-eFluor 710	482	710	710/50	685		
PerCP-Vio700	482	704	710/50	685		
PE-Texas Red	496/565	603	610/20*	600*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
PE-Vio770	496/565	770	780/60*	750*		If using 488nm Blue laser this filter combo is **NON**-standard; is standard for 561nm Yellow laser
pHrodo	560	585	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
PI	535	620	610/20*	600*		If using 488nm Blue laser, this filter combo is **NON**-standard, if using 561nm Yellow laser this combo is standard
POPO-3	534	570	586/15	-		
Pyronin Y	488	570	560/40*	545*		RNA. This filter combo is **NON**-standard for 488nm Blue laser
Qdot	-	-	-	-		
Qdot 525	350;405	525	525/50	505		
Qdot 545	350;405	545	530/30;525/50	505		530/30 BP if using 355nm UV laser; 525/50 if using 405nm Violet laser
Qdot 565	350;405	565	530/30;525/50	505		530/30 BP if using 355nm UV laser; 525/50 if using 405nm Violet laser
Qdot 585	350;405	585	585/42*;586/15	570		586/42 BP if using 355nm laser, 586/15 if using 405nm laser. UV filter set is **NON**-standard
Qdot 605	350;405	605	610/20*	570/600		610/20 BP with 570LP is **NON**-standard on UV laser; with 600LP is standard on 405nm laser
Qdot 655	350;405	655	660/20*	630*		If using 355nm UV laser this filter combo is **NON**-standard; is standard for 405nm Violet laser
Qdot 700	350;405	700	710/50*	690*		If using 355nm UV laser this filter combo is **NON**-standard; is standard for 405nm Violet laser
Qdot 705	350;405	705	710/50*	690*		If using 355nm UV laser this filter combo is **NON**-standard; is standard for 405nm Violet laser
Qdot 800	350;405	800	780/60	750		
RFP	563	582	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
RFP dTomato	554	571	586/15	-	575/25 BP with 550LP	Alternative filter set only to be used if using 488nm Blue laser for excitation
RFP tdTomato	554	581	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
Sapphire	399	511	525/50	505		
SNARF	548/579	587/635	586/15;610/20			pH 6/9; emission wavelength will depend on pH
Super Bright 436	414	436	450/50	-		
Super Bright 600	414	600	610/20	600		
Super Bright 645	414	645	660/20	630		
Super Bright 702	414	702	710/50	690		
SYBR Green	497	520	530/30			DNA
SYPRO Red	544	619	610/20	600		
SYTOX Blue	431	480	450/50	-		DNA
SYTOX Green	504	523	530/30	505		DNA
SYTOX Orange	547	570	586/15	-	575/25 BP with 550LP	DNA. Alternative filter set only to be used if using 488nm Blue laser for excitation
Tag-It Violet	395	455	450/50	-		
tdTomato	554	581	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
Texas Red	589	615	610/20	600		
TO-PRO-1	509	533	530/30	505		
TO-PRO-3	642	661	670/14	-	670/30	

TOTO-1	509	533	530/30	505		
TOTO-3	642	661	670/14	-	670/30	
TRITC	547	572	586/15	-	575/25 BP with 550LP	Alternative filter set only to be used if using 488nm Blue laser for excitation
UV1	-	-	-	-		
UV2	-	-	-	-		
V450	404	448	450/50	-		
V500	415	500	525/50	505		
Venus	515	528	530/30	505		
VioBlue	400	452	450/50	-		
VioBright FITC	495	519	530/30	505		
VioGreen	388	520	525/50	505		
Violet1	-	-	-	-		
Violet2	-	-	-	-		
Vybrant DyeCycle Green	488	534	530/30	505		
Vybrant DyeCycle Orange	488;532	563	530/30	505	560/40 BP with 505LP	
Vybrant DyeCycle Ruby	488;633	686	710/50;670/14	685; -		
Vybrant DyeCycle Violet	355;405	437	450/50	-		
X-Rhodamine	570	576	586/15	-	585/42	WARNING: use this wider BP with care: ↑ spectral overlap with other dyes in 610/20BP
YFP	514	527	530/30	505		
Zombie Aqua	394	516	525/50	505		
Zombie Green	496	515	530/30	505		
Zombie NIR	709	746	780/60	750		
Zombie Red	599	624	610/20	600		
Zombie UV	370	459	450/50	-		
Zombie Violet	398	423	450/50	-		
Zombie Yellow	398	572	525/50	505	610/20 BP with 595LP	