BD accuri C6 cytometer manual

Getting ready

Ensure that the fluid levels in the Sheath, Cleaning, and Decontamination bottles are sufficient to cover the inlet tubing and that there are no "kinked" fluidic lines.

Turn on the computer and log in with your UCF info. Start the Accuri CSampler software.

Press the Power button on the BD Accuri C6 instrument face panel. The instrument will begin a 5 minute auto start cycle. If the BD Accuri software displays the message *Extra Start-up time needed due to cleaning or improper shut down* it will take the instrument several more minutes for the instrument to get to the green-light ready state. Dripping from the SIP at the beginning of the Auto-Start cycle is normal.

When the instrument is ready, the traffic light within the workspace will turn from yellow to green. It is recommended that you install a tube of DI water and run in Fast mode or custom 100µl/minute mode for several minutes to verify fluid uptake and check for contamination with particles. If you are the first user of the day, you can run for 15 minutes; by then the lasers will be warmed up. Handle the SIP and Sample Support Arm Gently! It is also recommended to press the **Backflush** button and collect flow-out on kimwipes or into a tube (ca.100-200µl). The accuri is now ready.

If doing rare event detection or working with sticky samples, it is recommended that a *Backflush* is run between samples. Wiping the SIP and back flushing between samples will prevent carryover between samples.

Setting Fluidics Rate

The system can accommodate an upper limit of 10,000 events per second, but it is recommended to acquire samples at a rate of 2,500 events per second or less to ensure the best data resolution and prevent problems with clogging.
To set the fluidics rate: Select from the Slow(14µl/min), Medium(35µl/min), or Fast(66 µl/min) button in the Fluidics section of the Collect tab.

Threshold

Use thresholds to eliminate light scatter and/or fluorescence signals caused by debris in samples and electronic noise inherent in the system. By default, BD Accuri C6 Software is set to a primary threshold of channel 80,000 on FSC-H. Use the THRESHOLD button on the workspace. Threshold settings can be changed before, during, or after data acquisition. All thresholds are set on the Height signal for any given parameter.

- Width parameter is based on Threshold parameter setting
- FSC Threshold should be set from 200K-500K for most mammalian cells with the exception of very large cells 0.5M-1.0M or platelets (10K-30K). Begin at a value low in the range and raise as needed.

Setting a Run Limit

The run limit defines when data acquisition will stop. *Time*, *Volume*, or *Number of Events* in a specified gate are used to define the run limit. If multiple run limits are set, data collection stops on whichever limit is reached first. If the *Run Unlimited* check box is checked the sample will not stop running until 1 million events (the maximum allowed per sample) are collected.

Creating and Editing Plots

Create a plot(s) using the buttons located in each designated plot area in the Accuri workspace. Plots cannot be resized.
Use the Plot Spec Tool found in each plot to manipulate the data display in a plot, including axis parameter selection, channel range specification, and selection of linear or logarithmic axis scale. Set up or modify plot specifications at any time before or after collecting data.

To modify the event number to be displayed, Select **Display>Events Display Settings** from the top menu. Choose from All, custom number or custom percentage.

To re-name plot axis labels, click on a plot axis and Choose **Rename Parameters** from the dropdown menu. Enter a new name in the **Rename to** field.

Statistics

When a plot is selected, statistics for that plot and any gates drawn in that plot will show up at the top of the plot analysis area. You can select for Median Statistics to be revealed or hidden at anytime by clicking the Display tab > Show Median Statistics.

Gating

There are a number of gating types available. These include quadrant, rectilinear, and polygonal for 2-parameter plots and vertical and horizontal markers for histograms. BD Accuri C6 Software automatically displays the percentage of cells within the region.

- Gates cannot only be deleted from the workspace by deleting the plot on which it was drawn. Gate names are assigned sequentially by type (R1, R2 R3, P1, P2, P3, M1, M2 etc). as created.
- Custom gate names can be entered by double-clicking on the label and typing a new gate name in the dialog box.

Compensation

Compensation is the process used to correct for the spillover of fluorescence into the wrong detector(s). On the Accuri C6, all compensation is performed manually. Post- acquisition compensation can be performed using Accuri C6 software or third-party applications.

To perform compensation,

1. Open the Compensation Template from the desktop
2. Collect at least 500 positive events for each single-stain control
3. Adjust compensation manually using the Set Color Compensation dialog box. In the row associated with the detector to correct, click on the FL button of the fluorochrome channel that is creating the spillover. Adjust the value while monitoring the quadrant statistical means for the plot containing both FL readouts. Repeat this with all fluorochrome detector combinations in the experiment.
4. Apply the compensation values to all sample tubes.

Clogs/ Obstructions

The BD Accuri C6 has a 1-minute UNCLOG cycle which may be run any time an obstruction is suspected. Place an empty tube on the sample port and select the UNCLOG button in the workspace. Fluid should accumulate in the tube. The cycle may be repeated until fluid is observed in the tube.

Exporting Data

Data can be exported from sample wells as individual FCS data files. Files will automatically be saved to a folder named EXPORT on the desktop. Transfer a copy of the files to your UFCR server space and move them to your lab folder in the My Documents folder. Choose File > Export FCS File. Delete any old data from your folder at each visit.

Basic features of the BD accuri

Optics

Laser Excitation
488 nm (rated at 20,000-h life)
640 nm (rated at 20,000-h life)

Laser Profile
10 x 75 μm

Light Scatter Detection
Forward (0°, ±13°)
Side (90°, ±13°)

Emission Detection
4 colors, user-changeable optical filters

Standard set installed:
- FL1 533/30 nm (eg, FITC/GFP)
- FL2 585/40 nm (eg, PE/PI)
- FL3 > 670 nm (eg, PerCP, PerCP-Cy5.5, PE-Cy™7)
- FL4 675/25 nm (eg, APC)

Optical Alignment
Fixed alignment

Fluidics

Flow Cell
200 μm ID quartz capillary

Minimum Detectable Particle Size
0.5 μm

Minimum Sample Volume
50 μL

Pre-Set Flow Rates and Core Sizes
Slow : 14 μL/min, 10-μm core
Medium: 35 μL/min, 16-μm core
Fast: 66 μL/min, 22-μm core

Custom Sample Flow Rates
10–100 μL/min

Custom Core Diameter
5–40 μm

Recommended Sheath Fluid
0.2-μm filtered DI water

Maximum Events Per Sample
1 million events per well

Performance

Fluorescence Sensitivity, MESF*
FITC < 150; PE < 100

Scatter Resolution
Resolves human peripheral blood lymphocytes, monocytes, and granulocytes

Fluorescence Linearity
2 ±0.05% for chicken erythrocyte nuclei (CEN)

Fluorescence Precision
<3% CV for CEN

Data Acquisition Rate
10,000 events/second, maximum

Cleaning the instrument after use

1. When Cytometer is at green-light ready state, place a paper towel or tube underneath the SIP. Click **BACKFLUSH.**
2. Clean the "home position" of the SIP, i.e. the white receptacle on the plate platform: put Kimwipes around the "home position" and spray some water into the opening. Dry excessive water with more Kimwipes.
3. Place 4 tubes into the 24 tube rack: a tube with 2 mL of diluted **Decontamination** Solution, a tube with 2ml **diH2O**, a tube with 2 mL of diluted **Cleaning** Solution and another tube with 2 mL **diH2O** on the 24 tube rack. Select the data wells. Set time limit for **2 minutes** and Fluidics speed to **Fast**. Click **RUN** The Cytometer will stop automatically when the time limit is reached. Leave the tubes on the 24 tube rack until the Cytometer is used again.
4. Switch instrument off if nobody else will use it the same day. The BD Accuri C6 automatically runs a fluidics system shutdown. Depress the **Power** button on the Accuri instrument face panel for 1 second and the instrument will auto shutdown. Once the shutdown cycle has begun (Flashing blue light on front instrument panel) the computer can be powered off.
5. Please logoff Windows before you leave.

Taking care of fluids:

Empty the waste tank daily or when prompted by CFlow software to prevent spillover and possible biological safety risk.
1. Disconnect the waste level sensor and quick disconnect from the top of the Waste Bottle.
2. Remove the lid.
3. Dispose of waste according to local regulations.
4. Add approximately 100 mL of 0.5% NaOCl to the bottle.
5. Replace the lid.
6. Reattach the waste level sensor and quick disconnect to the top of the bottle.

Filling the Sheath, Decontamination, and Cleaning Solution Bottles
Before turning on the C6 Flow Cytometer, visually check all the bottles and fill the Sheath, Decontamination, and Cleaning Solution Bottles accordingly (instructions on how to prepare the solutions are found on the stock solutions).
1. Disconnect the appropriate level sensor and quick disconnect from the top of the bottle.
2. Remove the bottle lid.
3. Fill the bottle with appropriate reagent.
4. Replace the lid.
5. Reattach the level sensor and quick disconnect to the top of the bottle.
The C6 Flow Cytometer is a non-pressurized system. If needed, any of the bottles can be opened while the C6 is powered on.

Area	Recommendations
Sample concentration	1,000–5 x 10^6 cells/mL
Cell suspension	Assess and minimize cell clumping.
Sample medium	Calibrate fuidics when necessary to account for liquid viscosity.
Sample type	Cell lines, Primary cells, Beads, Bacteria
Sample volume	12 x 75-mm tube: 300 µL–2 mL Users should verify other tube/plate types, calibrate fuidics when necessary.
Fluidics speed	Standard settings: Medium or Fast Custom settings: Minimum settings are listed below. Appropriate flow rate and core size combinations are experiment specific and should be validated by the user.
Sheath fluid	0.2µM filtered water with bacteriostatic added.

Recommended preventive maintenance procedures:

Frequency	Task	Supplies and parts
After every experiment	Run filtered DI water at the Fast rate for 3 to 5 minutes to prevent clogging.	DI water
Daily (if the cytometer is not shut down)	Run the decontamination and cleaning cycles.	DI water, Cat. Nos. 653154, 653155, and 653157
Between uses	Place a tube of DI water on the Sample Introduction Probe (SIP), or if using the BD CSampler® option, place the SIP in the wash station.	DI water
Monthly	Clean the flow cell by performing an extended flow cell clean.	Cat. No. 653159
Bi-monthly	Replace the peristaltic pump tubing, in-line sheath filter, and bottle filters.	Cat. Nos. 653146, 653148, and 653147

Sample Preparation

Volume measurements on the BD Accuri C6 are most accurate with cell concentrations between 1,000 and 5000000 cells/mL. Higher concentrations might result in inaccurate counting due to system saturation, so dilute samples if necessary. The ideal cell concentration range for accurate counting varies among cell types, based on considerations such as size, shape, and tendency to clump. The flow rate, relative to sample concentration, should minimize doublets and larger clumps, **yet never exceed 10,000 events per second**. We recommend that you test serial dilutions of the sample and compare reported concentrations to verify a linear correlation.

Assess and minimize cell clumping, either by dilution or a combination of enzymatic and mechanical means. Cells particularly prone to clumping may need to be filtered prior to running on a flow cytometer. Cells must be evenly dispersed throughout the suspension; otherwise, no method can achieve accurate counting.

To assess cell clumping plot FSC-A against FSC-H. When single cells in the flow cell pass through the laser beam, their FSC-A and FSC-H signals correlate linearly and plot along a relatively straight line. Clumps of cells will have larger FSC-A signals relative to FSC- H, and the signals will fall off the diagonal formed by single cells. Diluting samples may help to reduce the percentage of clumping cells.

Useful tools for dye/fluorochrome selection:

http://www.bdbiosciences.com/us/s/spectrumviewer

https://www.thermofisher.com/us/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html?gclid=CPaFz7T_gdICFYEYgQodgywO7w&s_kwcid=AL!3652!3!81938021711!b!!g!!%2Bspectral%20%2Bviewer&ef_id=WFLtnwAABSMAgxpg:20170209024442:s

Table: Settings for various fluorescent dyes

Laser	Detector	Fluorochrome	Notes
488	FL1 530 BP	FITC GFP / eGFP/YFP/mCitrine CFSE/CFDA Alexa Fluor 488 Fluo-3 Qdot 525 JC1 DCFH	 Membrane potential, Mitochondria
	510 BP	eGFP	
488	FL2 585 BP	PE PI (propidium iodide) dsRed, dTomato, OFP SNARF TMRM Acridine Orange Qdots 585/ 605/ 665 Tetramethylrhodamine JC1	 pH indicator Mitochondrial activity Membrane potential, Mitochonria
	540 BP	eYFP, mCitrine	
	565 BP	OFP	
488	FL3 670 LP	PerCP PerCP-Cy5.5 PE-Cy5.5 PE-Cy7 PE-CY5 Red613/PE-TxRd Q Dot 705 7AAD / PI mCherry/RFP Mitotracker Red	dim signals may be a problem BEWARE! Lots of bleed into FL4 (may overwhelm dim APC signals)
	610 BP	PI/PE-TxRd	
640	FL4 675 BP	APC Alexa Fluor 647/660/700	
	780 BP	APC-Cy7	

BD Accuri™ C6 Flow Cytometer
Optical Filter Guide

Dyes	Laser	Standard Filters	Detector	Optional Filters[2]
Recommended optical choices for common fluorophores				
FITC, BD Horizon Brilliant™ Blue 515, Alexa Fluor® 488, CFSE	488 nm	533/30	FL1	—
GFP[3]	488 nm	533/30	FL1	510/15
YFP[3]	488 nm	533/30	FL1	540/20
PE	488 nm	585/40	FL2	—
OFP	488 nm	585/40	FL2	565/20
PI	488 nm	585/40, 670 LP	FL2, FL3	610/20, 630/30[4]
BD Horizon™ PE-CF594	488 nm	670 LP	FL3	610/20, 630/30[4]
RFP[3]	488 nm	670 LP	FL3	610/20, 630/30[4]
PerCP, PE-Cy™5	488 nm	670 LP	FL3	675/25[5]
PerCP-Cy™5.5	488 nm	670 LP	FL3	—
PE-Cy™7	488 nm	670 LP	FL3	780/60
7-AAD	488 nm	670 LP	FL3	675/25, 630/30[4]
APC, Alexa Fluor® 647	640 nm	675/25	FL4	670 LP[6]
APC-H7, APC-Cy7	640 nm	670 LP	FL4	780/60[5]

**Examples for DNA dyes on the BD accuri:**
Using the blue/488 laser
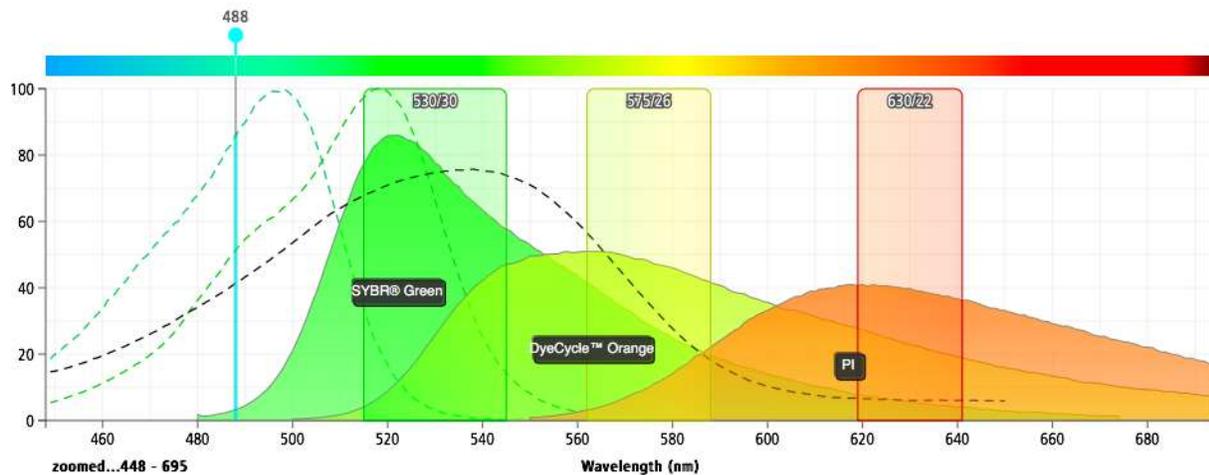

DNA dyes for the red/640 laser:
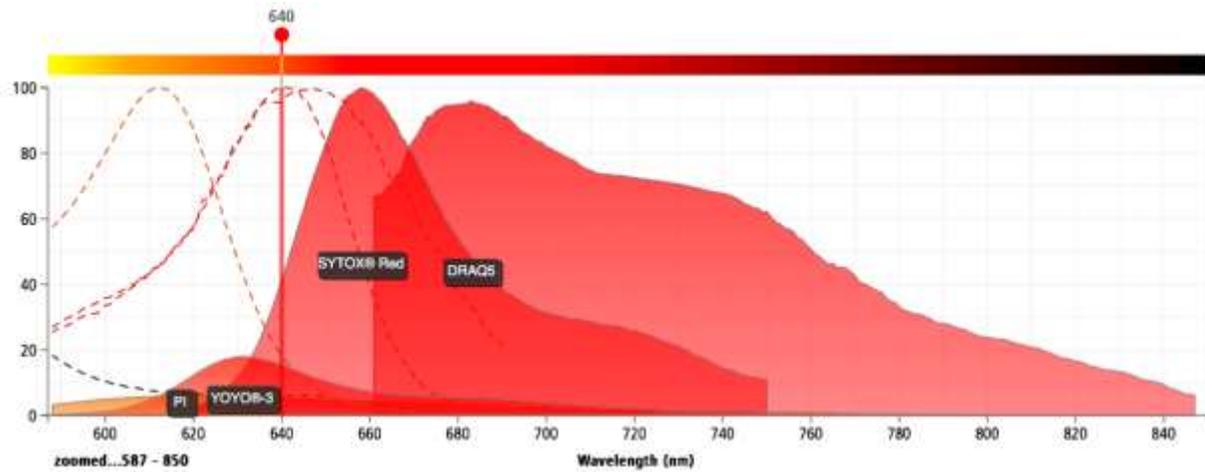
**Fluorescent proteins on the BD accuri:**
Blue laser:
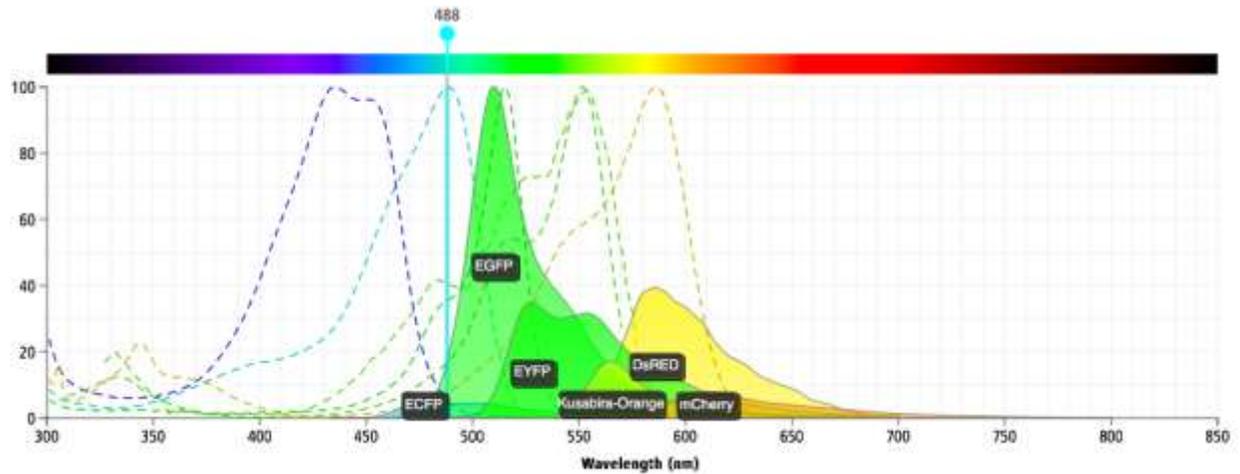
Red laser:
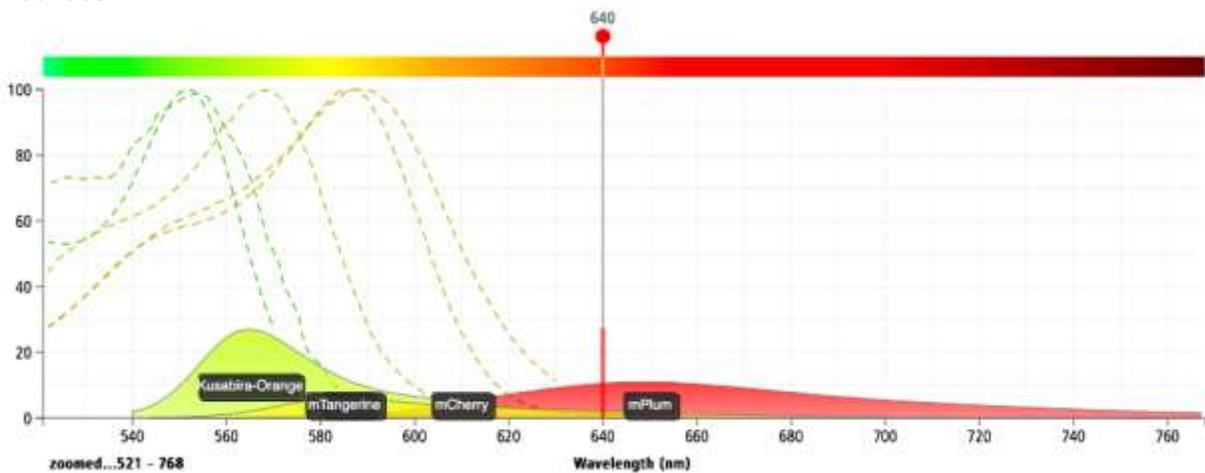
Examples of fluorochromes for the BD accuri:

Blue laser
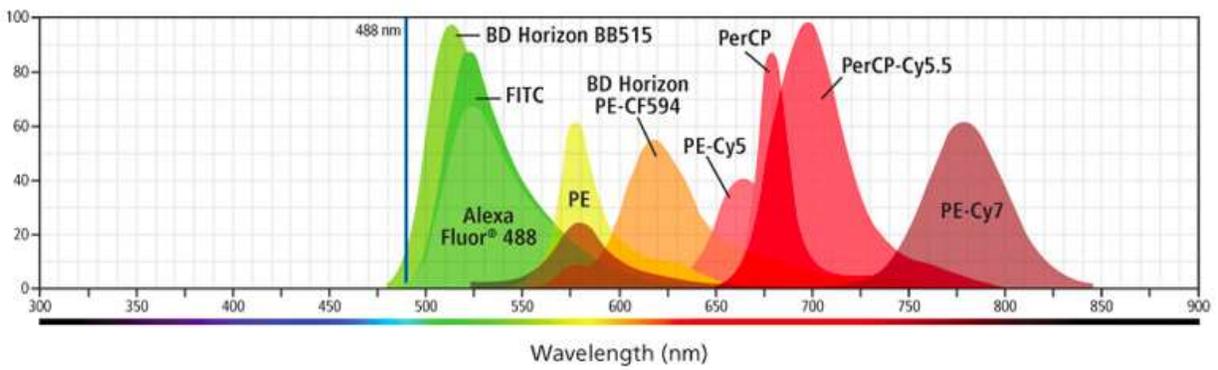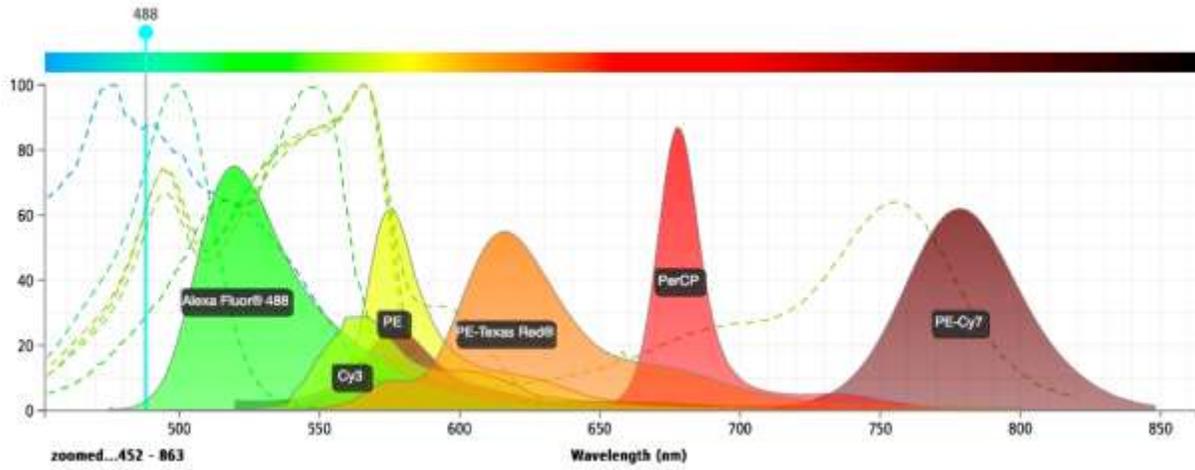
dyes for the 640/red laser:
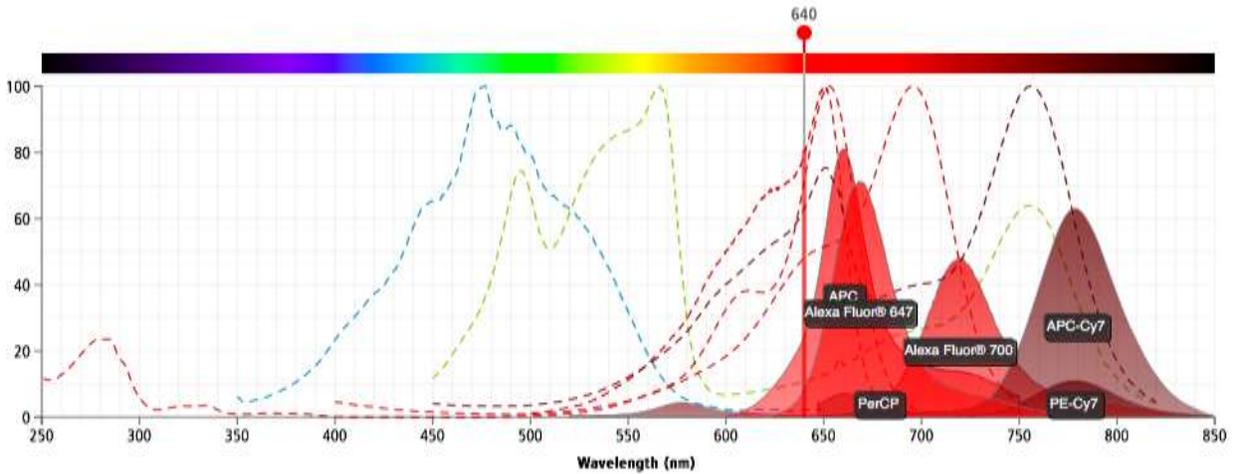
same dyes when activated by the 488/blue laser:

zoomed...437 - 850

Wavelength (nm)

Wavelength (nm)