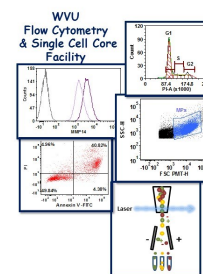


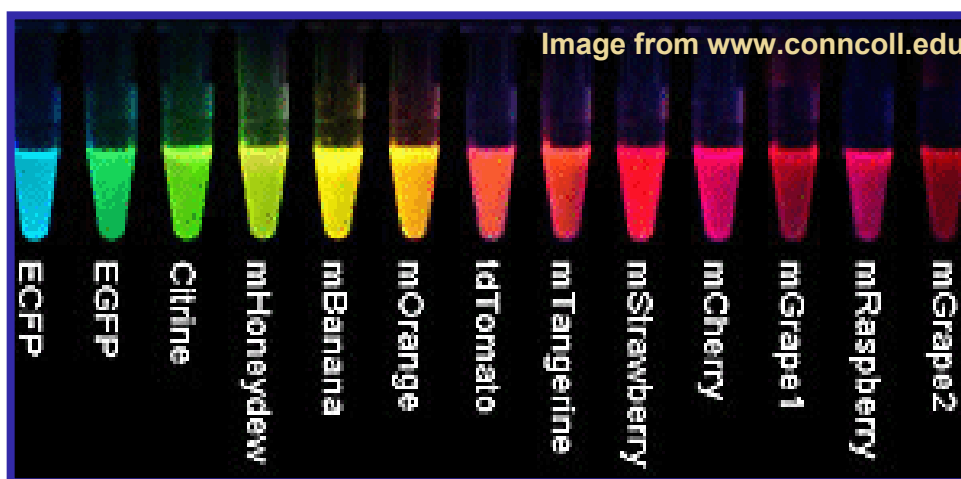
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 3, issue 2

October 2016

Choosing The Best Fluorescent Protein For Your Experiments



In 2008, the late Roger Tsien (1952-2016) was awarded the Nobel Prize in Chemistry for his work on green fluorescent protein (GFP). The multitude of fluorescent proteins (FPs) available to us for use in our studies is largely due to the work he and his lab has done over the years. With so many different FPs to choose from, the question becomes how do you pick the right one for your application?

General Requirements

Listed below are some general rules for selecting the best FP for your experiments. Some of these rules are common sense while others may be new to you.

1. The FP when expressed should not be toxic to the cell.
2. The FP should be bright enough to be easily detected above background autofluorescence.
3. The FP should be photostable for the duration of the experiment or have low photobleaching.
4. If expressing the FP as a fusion protein select one that does not oligomerize
5. The FP should not be affected by environmental conditions in the experiment particularly inside the cell.
6. If multiple FPs are going to be used in an experiment make sure to use ones that will have minimal spectral overlap.

Brightness

Whenever you do an experiment involving fluorescence you always want the fluorochrome/FP you use to be as bright as possible so that it is easily detectable above background. With FPs, the brightness of a given FP depends on a number of factors. Some of these factors are related to

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Facility Location:
 2160 HSCN
 Phone: 304-293-6273
 e-mail: flow-core@hsc.wvu.edu

Hours of operation:
 9:30 am to 5:00 pm, M-F

After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

the intrinsic characteristics of the protein including maturation speed/efficiency, extinction coefficient and quantum yield. Other factors are related to how the instrument that will be used to detect the FP is setup. When it comes to comparing brightness among different FPs a word of caution: Do not rely on manufacturer's recommendations when it comes to brightness. Remember they are trying to sell you a product.

Photobleaching/Photostability

Photobleaching is the result of a photochemical modification of a dye resulting in the irreversible loss of its ability to fluoresce. FPs tend to have a lower rate of photobleaching compared to many of the commonly used small molecule dyes. Within a particular spectral class the photostability of FPs can be quite varied. There are a number of factors that can affect the amount of photobleaching that will occur during an experiment including the strength of the excitation signal and the length of time the FP is excited. Thus, when choosing an FP it is important to keep in mind what assays you will be performing with it.

Recommended FPs Based on Spectral Class

Cyan Class (CFPs)

Cerulean is the brightest CFP, folds efficiently at 37°C and is the best general purpose FP. The CyPET variant has a narrower emission peak and is good when doing Forster Resonance Energy Transfer (FRET) with YFP variant YPet. However, CyPet is expressed poorly at 37°C.

Green Class (GFPs)

In this class, the most common FP is EGFP because of its good photostability. A newer variant, Emerald, has been shown to work better than EGFP because it folds extremely efficiently at 37°C. However, Emerald FP bleaches faster than EGFP.

Yellow-Green Class (YGFPs)

mCitrine, Venus and YPet work well for most applications. They are better than the older EYFP. When using YGFPs in a FRET experiment, YPet should be used with CyPet

Orange Class (OFPs)

The brightest monomer in this class is mOrange. Unfortunately it is not very photostable. Additionally, it is not very stable in low pH environments. For these situations, mKO is a better choice.

Red Class (RFPs)

The best monomer RFP is mCherry. It works well for most experiments. An equally good RFP is tdTomato. It is a tandem dimer that is very photostable. tdTomato is a fairly large RFP but can be used as a fusion tag as long as its size does not interfere with the function of the attached protein. mStrawberry is probably the brightest RFP but it is not very photostable compared to mCherry or tdTomato.

Far-Red Class (FRFPs)

In this class, mPlum is the only one that is a monomer, bright and photostable. It is not as bright as other FP monomers. It is the one that should be used in experiments where you have multiple FPs and are trying to minimize spectral overlap.

Flow Cytometers in this facility

FACSAria Cell Sorter

Operator: Facility Staff

**Lasers: 488 nm Sapphire
633 nm HeNe
407 nm Violet**

Detection Parameters:

Forward Scatter

Side Scatter

Simultaneous detection of 11 fluorochromes

Applications:

Cell Sorting (Aseptic)

Cell phenotyping

Cell Viability

FISH, FRET, SPA

LSR Fortessa

Operator: User

**Lasers: 405 nm OBIS LX
488 nm Sapphire
561 nm Sapphire
628 nm OEM**

Detection Parameters:

Forward Scatter

Side Scatter

Simultaneous detection of 12 fluorochromes


Applications:

Cell phenotyping

Cell Viability

Cell Cycle analysis



FISH, FRET, SPA

To log in and reserve a flow cytometer, C1, AutoMACS, MSD or analysis computer, please point your browser to the following URL 



<https://cores-wvu.mis.vanderbilt.edu/login.cfm>

Other Instrumentation in this facility

<p><u>AutoMACS Magnetic Bead separator</u></p> <p>Operator: User</p> <p>Application:</p> <ul style="list-style-type: none"> Single marker (extracellular) sorting Depletion sorting 	<p><u>gentleMACS Octo Dissociator with Heaters</u></p> <p>Operator: User</p> <p>Application:</p> <ul style="list-style-type: none"> Dissociation of tissues into single cell suspension for culture or flow cytometry assays Homogenizes tissues for downstream molecular biology applications
<p><u>C1 Single Cell Auto Prep System</u></p> <p>Operator: User or Staff</p> <p>Application:</p> <p>Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.</p> <p>Downstream applications:</p> <ul style="list-style-type: none"> RNA seq DNA seq PCR <p>Format: 96 or 384 chambers per chip</p> 	<p><u>MSD Multi-Array Platform</u></p> <p>Operator: User</p> <p>Applications:</p> <ul style="list-style-type: none"> Detection of cytokines, cell signaling proteins Multiplexed assay design: (1-10 analytes/plate) Detection range: 1 – 10,000 pg/ml Sample volumes: 25 µl or less Assay Time: 4—6 hours depending on analytes being detected 

Fee Schedule (2015-2016)

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	\$33/h	\$50/h	\$74/h	\$14/use	\$10/use	No Cost	No Cost	\$10/sample
Non-WVU User	\$49.50/h	\$75/h	\$111/h	\$21/use	\$15/use	\$75/plate	\$225/plate	\$15/sample

New User Guide

Hands-on training for FACSCaliber, LSRFortessa and C1 Single Cell Auto Prep System is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting as well as data acquisition on FACSAria is by facility staff only.

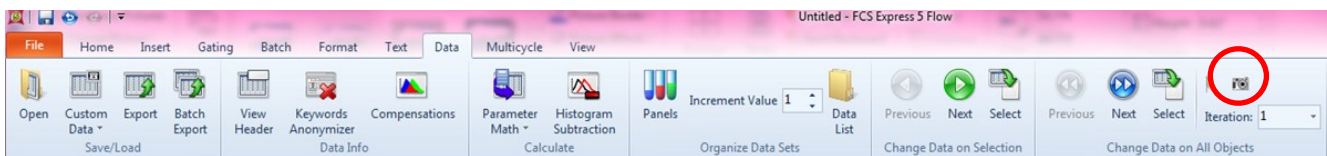
Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own.

FCS Express 5 Software: Snapshots Remembering Your Gate, Marker and Quadrant Positions

How many times have you positioned all your markers/gates/quadrants perfectly, done some additional analysis and realized you really want them back in the original position. You could keep hitting the undo button but that will only take you back so far. In FCS Express 5, there is a new feature called SNAPSHOT which allows the software to remember the positions of the markers/gates/quadrants when everything was perfect. There are 3 ways to use it:

1. In the layout you press the “camera” icon in the Data Tab (red circle below).
2. During a Batch Process you can apply a snapshot and re-run the batch process
3. You can define Manual Snapshots with descriptive names and apply them when you need them.

For more information visit the De Novo Software website (www.denovosoftware.com) and check out one of their tutorials.



Upcoming Holidays & Events

November 8, 2016	Election Day	Facility closed
November 23 - 25, 2016	Thanksgiving Holiday	
December 23 –17, 2016	Christmas Holiday	
January 2, 2017	New Year’s Day Holiday	
January 16, 2017	Martin Luther King, Jr Holiday	

Note to users:

Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):

FACSAria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

C1 System users: Experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers U54GM104942 (CTR), P30GM103488 (CoBRE) and P20GM103434 (INBRE).