# UCFlow

The University of Chicago Flow Cytometry Facility http://ucflow.uchicago.edu

TUESDAY, MARCH 18, 2008

## LSRII #2: Best Color Combos

Considering this LSRII will have some of the same lasers/filters as our other instruments, you may be thinking the color combinations will probably be the same. Well, you'd be right, but maybe not for the right reasons. Here, I'll explain the best color combos, and why.

Just for redundancy's sake, let's take a look at what's available. A 405nm with 3 PMTs, a 488nm with 2PMTs, a 561nm with 4 PMTs, and a 640nm with 3PMTs (4 lasers, 12 colors). So, let's say you want to do a 12 color experiment, which colors will you use.

Let's start with the blue. Off the 488nm laser you're options are going to be FITC, PerCP or PerCPCy5.5. Now, if you're going to use PECy5.5 off the YG laser, then you'll want to use PerCP off the Blue instead of PerCPCy5.5. If however, you will use PECy5 off the YG, then you'll want to use PerCPCy5.5 off the blue. Next, we'll tackle the red. Off the Red laser, your options are APC, APCCy5.5 or Alexa 700, and APCCy7 or APCAlexa750. We have a similar situation as before, you'll want to repeat "Cy5.5" as few times as possible. Additionally, you'll want to avoid repeating the same emission spectra off different lasers as much as possible. Cy5 and APC have the same emission, so you'd want to avoid using PECy5 and APC together. APCCy7 is not that great, so you probably want to opt for the APCAlexa750 option. Now, for the yellow-green (YG) line. Any of the PE and PETandems would be appropriate, so you'll have PE, PETexasRed, PECy5, PECy5.5, PECy7, PEAlexa610, etc... Again, pick emissions that you have not duplicated elsewhere. Finally, you have the violet. For the violet your choices are Pacific Blue, Pacific Orange, Qdots, or dyes like DAPI. Special care should be taken when choosing Qdots as most of the Qdots will be excited by the blue laser and maybe even the YG laser. They also have high quantum yields, so even if they get excited by a non-optimal laser line, they'll still be pretty bright. You should again try to use the Qdots in places where you have gaps in the emission of your other fluorochromes.

So, with all that said, let's pick a panel. I'm going to propose PacBlue, Qdot 565, Qdot 625, FITC, PerCP, PE, PEAlexa610, PECy5.5, PECy7, APC, Alexa700, APCAlexa750 for my 12 color assay. I chose the two Qdots because they fill the gap between FITC and PerCP, and are less likely to be excited by the YG or Red laser. This 12 color combination will offer the greatest sensitivity with the least amount of compensation requirements. If however, you need to look at fewer colors, you could envision a panel of maybe 6 or 7 colors requiring little or no compensation. Here's an example: Pacific Blue, Qdot 625, FITC, PerCP, PE, PECy5.5, APC. This 7 color panel would have little to no compensation necessary whatsoever. Pretty cool, eh?

Posted by UCFlow at 4:39 PM



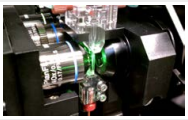
## 3 comments:



PAGES

- Home
- Disclaimer

FLOW CYTOMETRY



CONTRIBUTORS

- Ryan Duggan
- RynDggn

BLOG ARCHIVE

- ► 2012 (10)
- ► 2011 (19)
- ► 2010 (24)
- ► 2009 (24)
- ▼ 2008 (24)
  - ► December (2)
  - ► November (2)
  - ► October (1)

**Anonymous** July 16, 2010 at 2:50 PM

Thank you for this

Reply

**arumugam** December 21, 2011 at 3:18 AM

Wonderful blog & good post.Its really helpful for me, awaiting for more new post. Keep Blogging!

Blue Lasers

Reply

**Ryan Duggan** December 21, 2011 at 9:20 AM

Thanks arumugam. A post my longer diatribes here on the blog, but you can also read some of my stuf on our Facebook page. www.facebook.com/UCFlow

Reply

**Comment as:** Select profile...

Publish Preview

## Links to this post

Create a Link



Newer Post Home Older Post



**POPULAR POSTS**

Subscribe to: Post Comments (Atom)

Counting Cells with the EMD-Millipore Scepter 2.0

I recently had the chance to play around with the Scepter 2.0 Automatic Cell Counter from EMD-Millipore. The Scepter uses the Coulter Vol...

ISAC Wrap-up Part 1

Planning to submit a paper to J. Exp. Med. anytime soon? Well then you'll want to pay attention to the new guidelines generated by the Data...

EMD-Millipore 8HT Review

In my quest to find a mid-range cytometer to replace my ailing FACSCantos, I've come upon the 8HT from EMD-Millipore (whom I'll probably jus...