

## MODULE 10: CHOOSING FLUOROPHORES

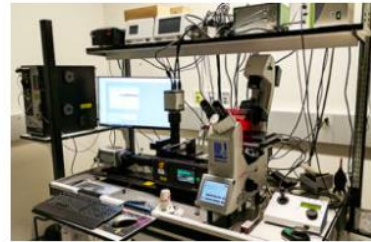
**Objective:** When planning an experiment, one should choose fluorophores that match the specifications of the microscope they intend to use. Specifically, excitation and emission specifications.

**Step 1:** Go to the facility website to check specifications for the microscope you wish to use.

<https://research.unityhealth.to/staff-services/research-facilities/facilities/imaging-facilities/imaging-equipment/>

### QUORUM SPINNING DISC WITH TIRF

- **Spinning Disc:** Discovery/Nipkow spinning disk with dual-pinhole sizes (50um & 100um)
- **TIRF:** Discovery TIRF Unit
- **Lasers:** solid state 405, 488, 561,637
- **Emission filters:** Dapi 450/50, GFP 525/50, RFP/Cy3 600/50, RED 610/75, Cy5 700/75
- **Objectives:** 10X(0.25), 20X(0.40), 63XOil(1.47; CORR TIRF)
- **Detectors:** Hamamatsu ImagEM X2 EM-CCD camera, and ORCA-Flash 4.0 V2 PLUS sCMOS camera
- [Book Now](#) (Room 778)



For the following steps, familiarize yourself with a fluorescence spectra viewer such as:

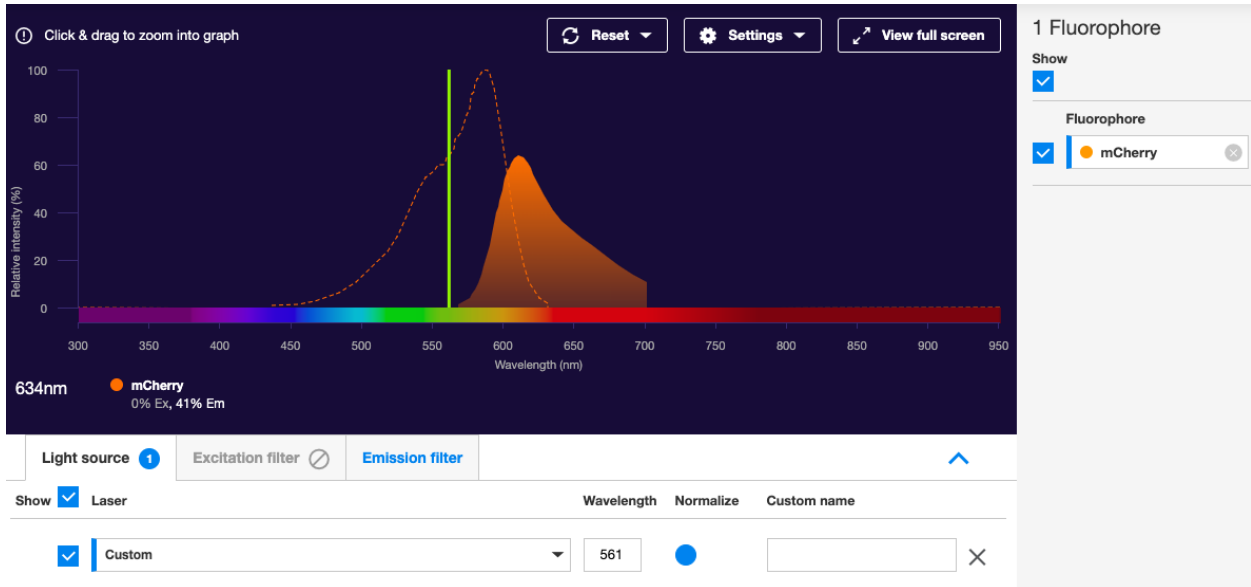
<https://www.thermofisher.com/order/fluorescence-spectraviewer#!/>

**Step 2:** Ensure the light sources sufficiently excite your fluorophores.

Example 1) 488 laser excites GFP



Example 2) 561 laser excites mCherry



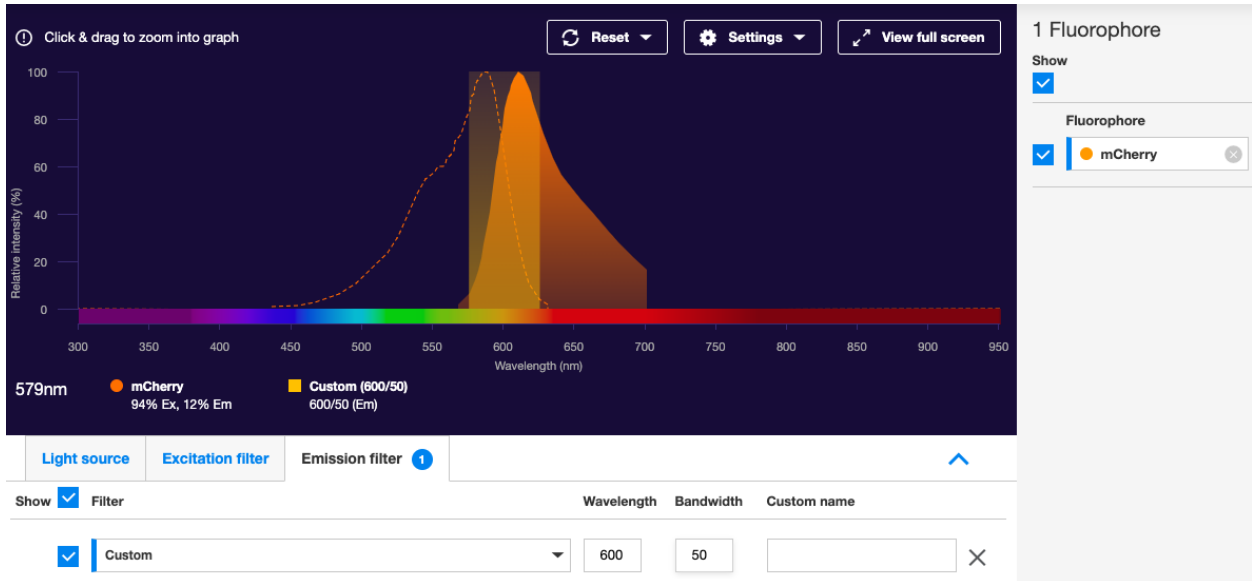
**Step 3:** Ensure the emission filters capture the emission spectra of your fluorophores.

- PRO TIP: Use spectra viewer to double check.

Example: GFP 525/50 emission filter



Example: mCherry 600/50 emission filter



**Step 4:** If using multiple fluorophores ensure they can be sufficiently separated.  
Example of good separation vs non separation:

**Example: Non-separation:** GFP / Alexa Fluor 514 using 488 laser, too much emission overlap



**Example: Good separation:** GFP/Alexa Fluor 555 using a 555 laser, less emission overlap.



For custom fluorophores please see imaging specialists.

## Typical combinations for each system

### 1. Typical 4 colour combination

- All systems except Olympus BX50 and Quorum Spinning Disc

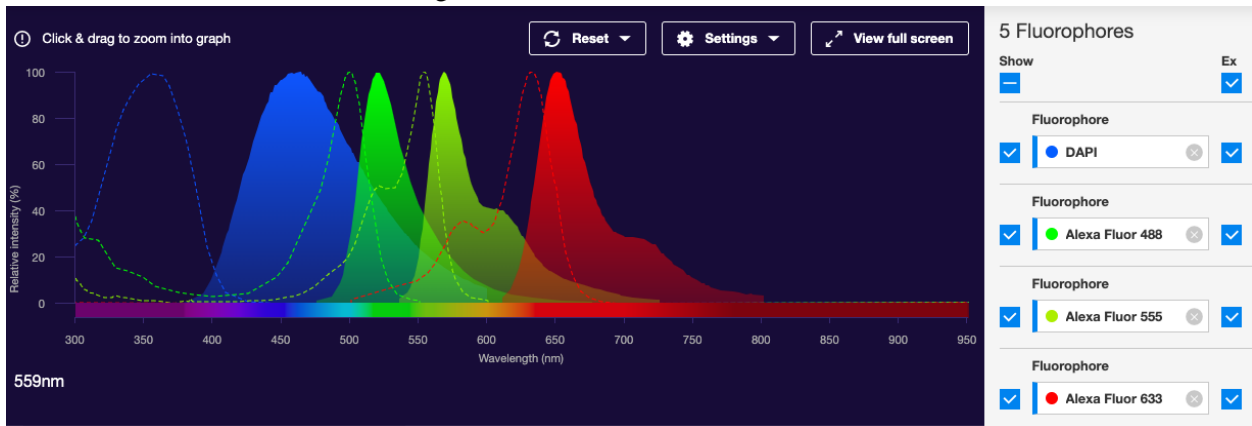


### 2. Quorum Spinning Disc 4 colour combination (has 730 laser)



### 3. Slide Scanner Combo 1

- More common slide scanning combination.



#### 4. Slide Scanner Combo 2

- Less common combination for slide scanning.

