STEM CELL ENGRAFTMENT
& IN VIVO ANALYSIS FACILITY

# T R O U B L E S H O O T I N G

**Open to comments from public (please submit to eks1@buffalo.edu)**

**Dapi stain emits at 488,**

**Q:**

We are staining with
DAPI and alexa-488 and get a VERY strange bleed through.

1. We illuminate with 488 and observe cytoplasmic staining (nothing in the nucleus).
2. We then illuminate with UV light from the HBO and observe a nice DAPI stain.
3. If we then re-illuminate with the 488 laser, the nucleus is now lit!

(these are PFA fixed cortical neuron primary cultures).
We have diluted DAPI to the point of losing the signal, and this still occurs.

WNYSTEM
NYSTEM

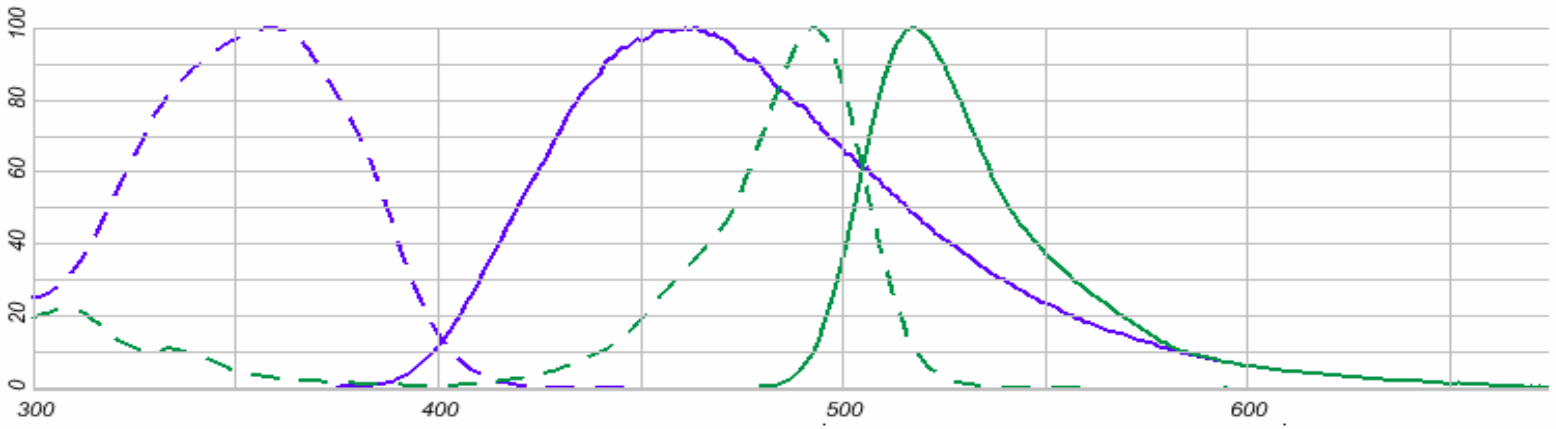
# TROUBLESHOOTING

## A:

**Interaction between emission from one fluorophore and excitation of another fluorophore -Most common**

**Cross-emission**

The emission spectra of two fluorophores overlap. Cross-emission typically happens toward the longer wavelength, as emission spectra have often long tails toward the right.




**Suggestion: to reduce cross-emission in multichannels acquisition, the 'reddest' dyes should be imaged first**

**Note: concerning crosstalk, DRAQ5 can be a good alternative to DAPI**