Flow Post-its
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Laser Delays

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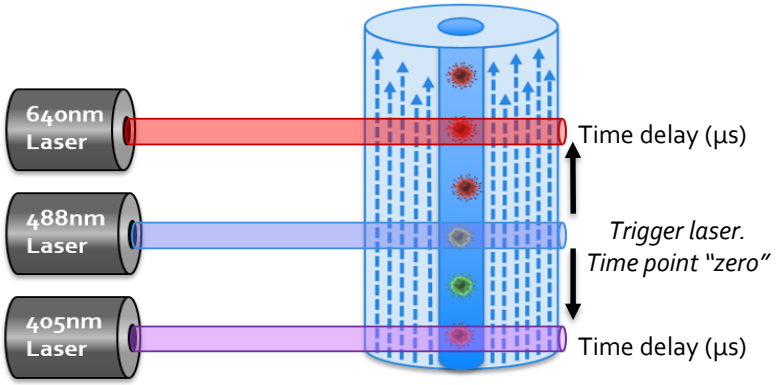
Most modern flow cytometers are equipped with multiple lasers to enable excitation of fluorochromes by different wavelengths of light. In order to discriminate emission from specific excitation sources, **lasers can be spatially separated**. To combine the signals emitted by the cell or event when passing through the lasers, the **laser delays**, measured in µs, must be calculated and checked routinely to ensure good quality data.



- **Laser delays are dependent on sheath pressure**. *Sample pressure does not impact laser delay*. Cytometer fluidic instabilities can lead to problems with laser delay/fluorescent signal and should be corrected.
- Daily QC should be done to check that laser delays are correct.
- Incorrect laser delays will result in erroneous population frequency; lower than expected Median Fluorescent Intensities (MFIs) and; poor post sort purity.
- **Not all instruments are equipped with spatially separated lasers**. Co-linear lasers are not separated by space/ time, therefore fluors that are excited by different lasers but with highly overlapping emission spectra (e.g., PE-Cy7 and APC-Cy7), cannot be analyzed simultaneously.

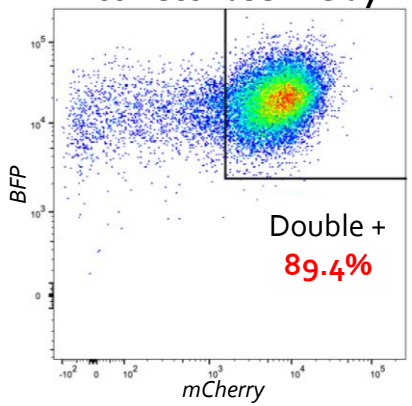
Laser Delay Changes: A Case Study

When setting up a cell sorting experiment, a researcher noticed that the population of BFP & mCherry double positive was not the same as expected or observed on an analyzer immediately prior to the sort.

Upon investigation, it was determined that the laser delay of the cell sorter was incorrect (left). This resulted in a tail of lower expressing /negative cells with only 89.4% double positive for mCherry and BFP.

Once the laser delay was optimized (right), the same sample was reacquired. After this was done, the double positive population resolved better at 97.6%.

Incorrect Laser Delay

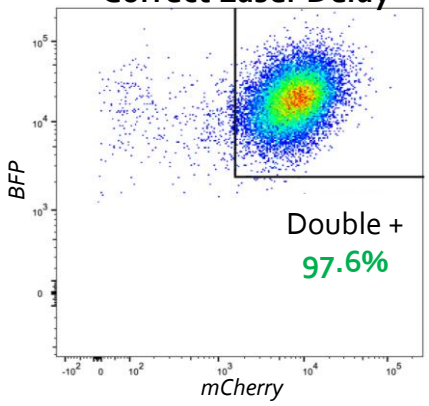


Incorrect Laser Delay

Yellow-Green Laser Delay: **-93.00 µs**

mCherry MFI of double+ population: **6867**

Correct Laser Delay



Correct Laser Delay

Yellow-Green Laser Delay: **-91.00 µs**

mCherry MFI of double+ population: **7685**