



Why Time Delay Calibration “Can Miss the Point... In time”

As the technology of flow cytometry advances, researchers and clinicians wish to perform assays using more colors. A method to accomplish these multi-color assays is having multiple laser excitation sources. In most cases these multiple laser sources are spatially separated, creating emission pulses from a single cell (event) to occur at different points in time. Time Delay Calibration is a feature of some dual laser systems with inter-laser compensation use to align these events in time.

Systems with this feature require a that the time delay between laser signals be precisely programmed into a variable delay line so a percentage of the blue laser signal can be subtracted from the delayed red laser signal using analog electronics. This programmed delay time must be accurate to within a fraction of a microsecond for inter-laser compensation to be accurate. Any drift in this delay time will result in under compensation, and a drift of FL3+/FL4- cells into the FL3+/FL4+ quadrant.

Factors affecting this delay time include sheath air pressure, the sheath tank going from full to empty, and drift in the vertical position of the red laser. Cytel’s Ultra Stable Fluid Manager System utilizes an ultra precise sheath regulator and a constant level sheath tank to stabilize the delay time between lasers.

Cytel’s Multi-Color upgrade do not use delay lines to attempt to “line up” the pulses between lasers. Instead, the FL3 pulse is held until the FL4 pulse arrives, and compensation is performed between two held or stretched peaks. The advantage of this is that timing delays can vary 10us without affecting inter-laser compensation, compared to a fraction of a microsecond for systems using delay lines. The disadvantage of this approach is that second laser parameters cannot be used as pulse processing parameters, and second laser parameters cannot be used as trigger parameters. However, given the danger of getting false FL4+ events due to under compensation, it was decided this was a reasonable tradeoff.