

PTI Technical Note

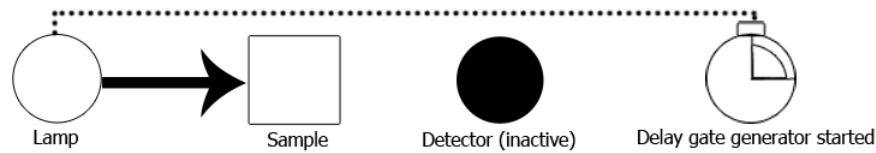
The Strobe Technique

PTI pioneered the strobe technique. The company has a patent on the instrumentation related to this technique. PTI is the only company that offers this technique for the measurement of fluorescence lifetimes. We have placed over 100 systems into service. The technique is not new, there are publications using it. It is an accepted and proven technique.

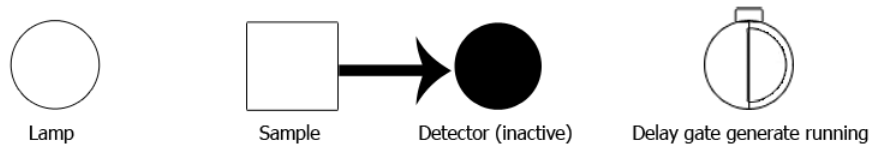
The major advantages of this technique are low cost, excellent performance and ease of use. No other technique offers these benefits for the measurement of lifetimes.

The major difference between the TCSPC and the Strobe techniques is in the detection. The same nanosecond lamp is used to generate the same excitation pulse. But now, the detector (which is a specially-configured PMT) is only active for a very short period of time after the excitation pulse is released. The detector records the intensity of the fluorescence at the moment it is active.

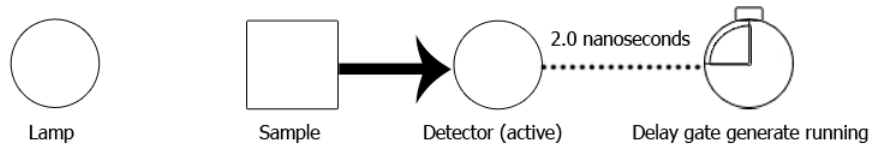
The window of time during which the PMT is active is controlled by a delay gate generator. The standard delay gate generator used with our existing systems steps the delay in 100 picosecond increments. The delay increases with each pulse (in other words, it sweeps the delay). In this manner a decay curve is produced. Here is a graphic representation of the Strobe technique:



1. The lamp pulser starts the delay gate generator and triggers the lamp.

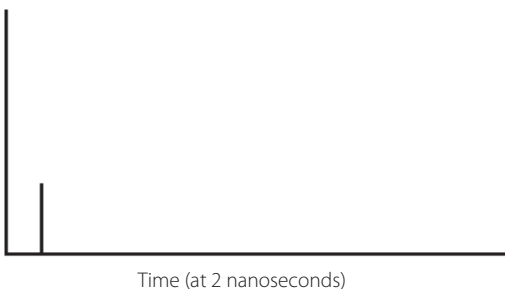


2. The excited sample begins emitting while the delay gate generator is running.

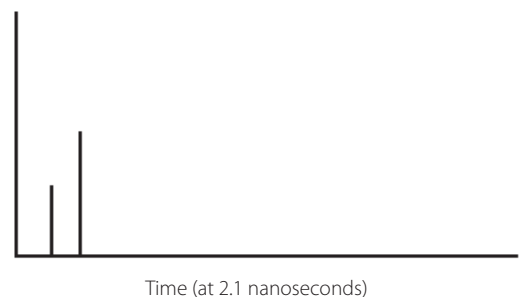


3. The delay gate generator activates the detector, which measures the emission intensity.

The three steps illustrated above are repeated. With each cycle, the delay gate generator increases the delay by a multiple of 100 picoseconds and the data is collected again.



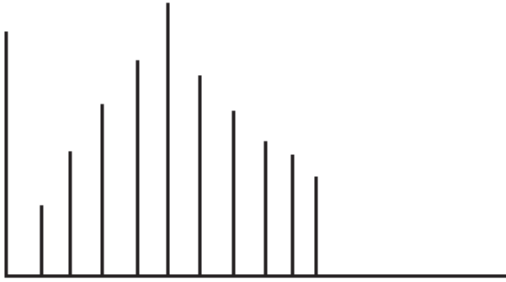
This is how the data looks after the first pulse.



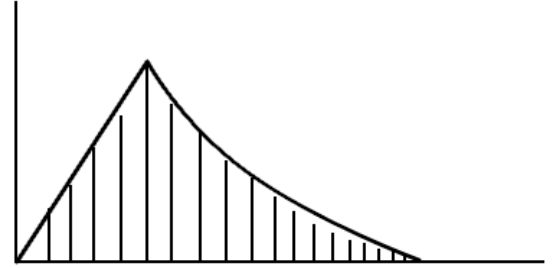
The second pulse is measured at 2.1 nanoseconds.

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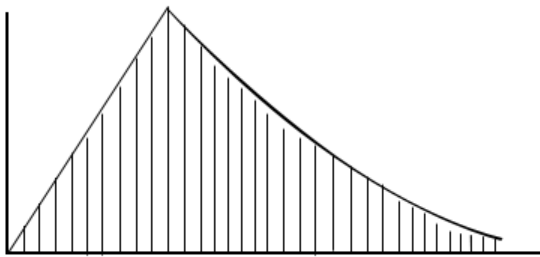


After ten pulses, the data begins to resemble a decay curve.

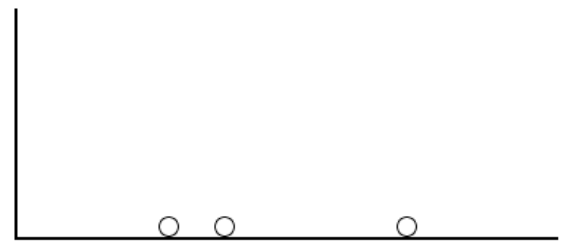


When all of the data is collected, the computer fits it to a model decay curve.

For increased accuracy, the entire sweep can be repeated, and the individual intensities averaged. This is helpful when the overall signal is low. However, as you can see, a full decay curve is attainable after just one sweep. Compare this with TCSPC: for every 100 pulses, you get only up to three useful statistical points to build up a decay curve. As shown below, the contrast is striking.



STROBE after 100 pulses



TCSPC after 100 pulses

The Strobe technique is much faster than the TCSPC technique for generating the decay curve. This is particularly important in the life science area. Whereas the chemist can take hours or days to measure an inert chemical very accurately, the life scientists' cell samples are long dead. In fact, this is one of the reasons why the Phase technique has survived, even though is inferior and complicated – there were simply no other alternatives for life scientists.

The Strobe technique has an additional feature, which allows measurement of a single lifetime almost instantaneously (10 to 15 seconds).

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