Photon Technology International

Comparison of the Lifetime Techniques

Within each technique there are different categories of systems that can be built. These systems can have vastly different performance characteristics and address different, sometimes very specialized applications. At the expense of hundreds of thousands of $$ a company can built a system that can measure a lifetime of 10 ps or less, but it can be next to useless for many other applications. On the other extreme, at a cost of 5-10K, one can build a simple device that will measure lifetimes in the neighbourhood of 10 ns at a fixed excitation wavelength addressing a single application. Although PTI has expertise to address such specialized needs and can provide highly customized systems, ***the main goal of our lifetime instrumentation is to satisfy a broad range of possible applications.***

The main market for lifetime instrumentation is still an academic market, although there is a growing demand from industries, especially pharmaceutical and from companies developing various solid state devices. Instrumentation that is available from PTI and its competitors usually falls in the ***price range of ca. 50K to somewhat over 100K***. There are certain important features that an instrument must possess in order to be useful for a broad range of applications:

Excitation wavelength

Even within a single application one may need to vary an excitation wavelength over a broad range. Protein research is probably the most common single application. The same researcher may study a protein using intrinsic native aminoacids, tryptophan or tyrosine, which require UV excitation (260-300nm), but may also want to introduce an external fluorescent probe, e.g. fluorescein or rhodamine derivative, that requires an excitation from 480 to 600 nm. An industry that develops colour screen technology may need to excite blue, green and red phosphors from the UV to the red spectral range.

In all these cases ***where the excitation wavelength coverage is of importance, PTI really excels over competitors***. We have developed a unique stroboscopic technique that can utilize and is designed to use ***low repetition lasers***, especially the one that PTI offers: the ***nitrogen/dye laser*** with an optional ***frequency doubler***. None of other techniques, TCSPC or phase, can utilize such a light source. The result is excellent wavelength coverage (245-990nm) with a single laser source and very high sensitivity throughout this entire range. For less demanding applications and for those on a tighter budget, a nanosecond flash lamp (NanoFlash), a medium frequency (up to 25KHz) light source is available.

The TCSPC technique is limited to what it can utilize as light sources. The technique suffers from its intrinsic inefficiency, as it can only accept no more than ***3 photons*** out of ***100 photons*** emitted, so it needs really ***very high repetition sources***. The traditional light source is a nanosecond flash lamp, which is the same type of source as PTI NanoFlash lamp, with the same wavelength limitations. Other choices are: ***mode-locked lasers*** (typically argon-ion and Nd:YAG with dye lasers and frequency doubler), expensive, unstable, difficult to maintain and providing very limited wavelength coverage; ***diode lasers*** which are not very expensive, but offer no UV and only one wavelength from a given laser head; and finally ***LEDs*** (light emitting diodes), very inexpensive, but limited with wavelength range and much weaker that diode lasers. For those with unlimited budgets there is a tempting choice of ***Ti:Sapphire laser***, but the price tag of the system (ca. 250K) really exceeds the merit of discussing it here.

The phase technique uses a ***continuous Xe arc lamp*** as a basic light source. The light source is modulated by an ***electro-optical modulator***. In principle, the Xe arc lamp offers very good wavelength coverage from UV to near IR. However, as mentioned in the previous chapter, the combination of the arc lamp and the electro-optical modulator represents a ***pinnacle of optical inefficiency***. As a result, more than 95% of the light energy is lost, degrading thus the sensitivity of the instrument. Continuous lasers, such as He-Cd, are sometimes used in order to improve the light transmission efficiency, but the wavelengths available are limited to 325nm and 442nm, which are rather useless for the most common applications. The phase can utilize the same very fast mode-locked lasers as the TCSPC does, but the complexity and the price tag for such systems, not to mention the wavelength limitations, make them very specialized items, not applicable for the comparison here.

To summarize: **The PTI LaserStrobe system is the best performer, the most versatile and the best value in the wavelength coverage category!!!**

Lifetime Range

This is probably the most misrepresented specification for a lifetime system, especially at the ***short lifetime range***. One must remember that the vast majority of fluorophores exhibit lifetimes from ***several hundred picoseconds*** to ***several hundred nanoseconds***. Most commonly used fluorescent probes have lifetimes between ***2 ns*** and ***20 ns***.

PTI lifetime systems cover the range from about 100 picoseconds to about 50 microseconds (laser-based) and from 100 picoseconds to about 1 microsecond (NanoFlash-based). **These ranges are more than adequate for most applications!**

One must remember that those who need to measure really very short lifetimes, from femtoseconds to few tens of picoseconds, will require sophisticated, expensive, custom-made setups. The standard systems from our competitors will not be suitable either.

Some of our phase competitors have been pushing the issue of short lifetimes by making claims that an arc lamp modulated phase system can measure 10 ps lifetime. **THIS IS NOT TRUE!!!** Refer to page 144 of the book Principles of Fluorescence Spectroscopy (2^{nd} edition) by Lakowicz. It clearly shows that even to measure a 100-ps lifetime, the gigahertz modulation frequency is required! Only fast mode-locked lasers and multi-channel plate (MCP) detectors (not PMTs, not arc lamps) must be used to obtain such high modulation frequencies. Again, these are expensive and complex propositions and are not comparable with standard systems under discussion.

Standard TCSPC systems show the same short lifetime capability as PTI strobe systems. Much more expensive systems equipped with pico/femtosecond lasers and MCPs can measure lifetimes down to a few tens of picoseconds.

PTI has an expertise and ability to provide a customized system for very short lifetimes. Since such systems usually have also other special requirements, the decision to offer such system is made on case-by-case basis.

Sensitivity

Comparing sensitivities is a rather difficult and speculative subject. In order to collect meaningful sensitivity data, one would need to gather all competing instruments in one lab and measure same weak samples under comparable condition. Although this is a rather unlikely scenario, some qualitative judgements can still be made.

If one were to compare a strobe system with a TCSPC system, both using the same NanoFlash lamp and the optics, the TCSPC would be an ultimate winner. After all, this is expected, as the technique is designed to measure single photons, i.e. the smallest quantities of light. In many cases however the practicality of collecting infrequent single photons from a very weak sample may be dubious, as the collection of a meaningful decay curve may take many hours or even days. The sample may deteriorate during this time or the instrumental instability may render the collected data useless. On the other hand, the same sample that can be barely detected with the flashlamp-based TCSPC, will produce a very strong signal in the LaserStrobe and the decay curve can be collected in minutes. In other words, since the ***strobe is the only technique that can utilize the low repetition, powerful, inexpensive nitrogen/dye laser, it makes the strobe the real winner over the conventional TCSPC system.***

The status of the phase technique is even more difficult to assess. Some past demo results indicate (based on a customer's assessment) that it took much longer to collect data for a certain sample in the visible range with a phase system than with our NanoFlash-based strobe. More currently, we've received some comparisons from a customer on tryptophan lifetime in a protein measured with the phase instrument from ISS and on PTI NanoFlash strobe and the LaserStrobe systems. ***It appears that the PTI NanoFlash system could measure 100x lower concentration and the LaserStrobe 10000x (!!!) lower concentration than the ISS phase!*** This comparative assessment is quite in line with the well-known inefficiency of the arc lamp/electro-optical modulator combination that results in very poor light transmission and degraded sensitivity of the phase technique.

The ***laser-based strobe system is the winner in the sensitivity*** contest. The various systems can be arranged as follows:

LaserStrobe > TCSPC > NanoFlash Strobe > Phase

Speed

In the old days, when the phase technique was using just a single frequency, it was definitely the fastest technique.

The ***multi-frequency phase is much slower than a single-frequency phase***, but there is no direct comparative data with the strobe. Some competitive advertising indicates that the ***modern multi-frequency phase is a rather slow technique***. One manufacturer (ISS) offers a ***FastScan upgrade*** to a regular system with the following statement: "…lifetime data acquisition on a routine sample can be acquired ***in one minute***…". Not very impressive, as without any "upgrade", PTI strobe can acquire data in less than 0.5 min and with strong emitters and a proper acquisition menu set-up, the task can be accomplished in ca. 5 sec!

It is quite obvious that the (conventional) TCSPC will be the slowest of the three techniques, when the sample is a strong emitter, due to the imposed requirement of collecting of only very small fraction of emitted photons.

With regard to the speed, ***PTI strobe is again the winner*** and various techniques can be rated as follows:

For strong to medium emitters:
NanoFlash Strobe > LaserStrobe > Phase > TCSPC

For weak emitters:
LaserStrobe > TCSPC > NanoFlash Strobe > Phase

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