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PhotoniQ: Data Acquisition Modes

Application Note

Although PhotoniQ multichannel PMT data acquisition systems are versatile laboratory instruments used for a variety of purposes, the systems are mainly designed and optimized for two types of applications, particle analysis and scanned imaging. These two classes of applications, which include bioaerosol fluorescence detection, flow cytometry, and confocal microscopy, share many similarities in the way their PMT signals are processed by the PhotoniQ. However, because the signals from particle analysis are essentially temporal in form and those from imaging spatial, each requires its own unique method of triggering and data acquisition. The PhotoniQ has two high speed acquisition modes to accommodate these applications. To take advantage of its considerable functionality and features, it is best to distinguish how each application generates data and how the PhotoniQ processes it.

PhotoniQ Signal Processing

The PhotoniQ signal processing functionality can be understood by exploring the similarities between particle and imaging applications. Both rely on the detection of very low level optical signals across one or more spectral channels. Usually the signals of interest are generated and measured using multispectral laser induced fluorescence detection techniques. The multianode PMT (MAPMT) together with the PhotoniQ provides the means to measure high speed optical phenomena produced by these techniques with single photon sensitivity. A grating or optical filter in front of the MAPMT separates the induced signal by wavelength so that each charge output from the separate PMT anodes corresponds to a different energy band from the incident signal. When triggered, the PhotoniQ independently processes each energy band by integrating and digitizing the signal charge. After some additional digital signal processing to improve uniformity and remove sources of error, the PhotoniQ organizes the data into an *event packet* that contains the PMT signal charge for each of the spectral channels. This *event packet* is output over the USB interface to a PC where it is displayed using the PhotoniQ graphical user interface, logged to disk, or further processed by a user's custom software application. Intrinsic to the design of the PhotoniQ is that each trigger generates exactly one *event packet* of spectral data only if the trigger is *accepted* by the system — not all triggers are accepted. The concept of one *event* for each *accepted* trigger is important for the understanding of the PhotoniQ's data acquisition modes.

Particle Analysis

Within the field of particle analysis are applications such as bioaerosol fluorescence detection, laser induced breakdown spectroscopy, flow cytometry, and multiangle light scattering spectroscopy. All of these share a common trait as it relates to data acquisition — the signals of interest are stochastic in nature. The science of particle analysis is often more concerned with the probabilistic distribution of particle types than with the absolute particle-type count. For the most part, this is important in analysis applications where an air or fluid column flows continuously past an array of optical sensors. Here, the emphasis is on getting an accurate representation of the particle-type distribution as a function of time. While it is usually advantageous to operate these systems as fast as possible, it is even more important that the data acquisition process be continuous over time with no large gaps in which no data is acquired. It is common in these systems for the trigger to the data acquisition unit to be generated by a separate light scatter channel focused on the particle stream. In this way the trigger rate can be controlled by adjusting the particle flow rate and the system triggered only when a particle is detected. Since particles in these systems typically exhibit interarrival time behavior that follows a Poisson distribution, where there is a non-zero probability that two particles can be spaced apart in a vanishing small interval, there is a likelihood that brief, extremely high triggering rates can occur. Recognizing this and the fact that no data acquisition system can operate at infinite speed, there will always be a situation in which some particles in the flow are missed. That being said however, performance can be optimized if the flow rate is adjusted so that the trigger rate of the data acquisition system yields the requisite percentage of particles acquired. This sacrifice is made for the sake of data acquisition uniformity and permits for real time processing of the acquired particle stream.

Scanned Imaging

Consider now the field of scanned imaging which includes fluorescence confocal microscopy. Compared to particle analysis where a flow of particles is uniformly sampled over time, scanned imaging involves a stationary subject that is scanned across a sensor using mirrors, usually in two dimensions, to produce individual pixels. While the resulting signal may appear to the data acquisition system to be temporal like in particle analysis, it is in fact simply a temporal representation of a spatial image. This fundamental difference between scanned imaging and particle analysis imposes the strict requirement that all pixels must be acquired by the data acquisition system. If not, the image will have blank spots in the locations of the missing pixels. The data acquisition system therefore must be triggered at a rate equal to the pixel rate and no triggers can be missed. The trigger generation is usually accomplished by synching it to the pixel clock from the mirror scanning electronics. Since a non-real time display of the subject image is acceptable in most situations, the large *snapshot* of data that is generated in the short period of time while the subject is being scanned can be buffered and then output to slower devices or applications over a longer period of time.

PhotoniQ Data Acquisition Overview

The user configures a PhotoniQ to acquire data by, amongst other things, selecting a trigger source and setting its acquisition mode. A detailed discussion of the PhotoniQ triggering capabilities is outside of the scope of this paper but it is worth mentioning that while some applications utilize the internal trigger modes such as *internal trigger* and *input trigger*, particle analysis and scanned imaging applications typically generate their own triggers and therefore use the external modes. Regardless of the source, a trigger to the PhotoniQ does not necessarily result in the acquisition of an event. It is necessary to understand this fact to get a true appreciation of the PhotoniQ's data acquisition capabilities.

The conditions under which a PhotoniQ will accept a trigger are strongly dependent on the data acquisition mode. Four acquisition modes are available in the PhotoniQ for the purposes of acquiring data. A fifth mode called *Log File Viewer*, is a pseudo-acquisition mode not used for acquiring data, but rather for viewing logged data that had been previously acquired. Of the four true acquisition modes, two modes, *Particle* and *Image*, are high speed modes that stream event data to the PC where it can be logged to disk or operated on by a user's custom software application. In a third mode called *Display Only*, event data is sent directly to the PhotoniQ's graphical user interface display — no data is logged or made available to other applications. The fourth true acquisition mode is essentially a combination of the *Display Only* and *Particle* modes and is appropriately called *Display & Log* mode because the unit logs all events that are displayed in the GUI.

In general, the two display acquisition modes are used to set up a system's optics and verify its setup. While the real-time display of event data makes for an excellent diagnostic tool, the significant overhead associated with refreshing the display limits the speed at which events can be acquired. For applications that require high speed data acquisition, either the *Particle* or *Image* modes should be used.

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PhotoniQ Data Acquisition Mode: Particle

It is recommended that in particle analysis applications, signals be acquired using the PhotoniQ *Particle* mode. In this mode the particle trigger clock supplied to the PhotoniQ can be at virtually any rate that the user chooses. The PhotoniQ however, will only accept the trigger clock and acquire an event of data if its internal data buffers are not full. Thus, no events will be missed if the following two conditions are met: no two consecutive triggers pulses occur in an interval less than the Minimum Event Pair Resolution (MEPR) specification, and the average event rate to the PhotoniQ is less than the Sustained Average Event Rate (SAER) specification for the unit. The MEPR requirement results from a hardware limitation at the front end of the PhotoniQ. Specifically, two consecutive events cannot occur any closer than the minimum period at which they can be digitized by the unit. The SAER requirement ensures that the average rate at which the PhotoniQ acquires events does not exceed the average rate at which it transmits events to the PC. In other words, the average event rate cannot exceed the bottleneck limitation at the USB interface to the PC. A small amount of buffering within the PhotoniQ permits short bursts of events that momentarily exceed the SAER to be acquired with no missed events, provided that no two events occur closer than the MEPR. Whenever any of the above requirements are not met, the PhotoniQ ignores the incoming trigger signal and therefore misses its corresponding event. Fortunately, the robust design of the unit allows it to perform as if the trigger signal never occurred. As a result, the PhotoniQ is armed and ready to accept the next event provided its corresponding trigger signal meets the aforementioned conditions relating to the MEPR and SAER.

The performance of the PhotoniQ in *Particle* mode is better understood by way of some examples. Consider first the case where two very closely spaced events occur at an infrequent repetition rate (relative to the SAER specification), and a trigger corresponding to each event is applied to the PhotoniQ. The system is setup to acquire an indefinite number of events by setting the *Trigger End Count* parameter to zero. If the time between events exceeds the MEPR, the two events will be captured one hundred percent of the time by the PhotoniQ. As the event spacing is adjusted to the point where it becomes less than the MEPR, the PhotoniQ will acquire every other event and consequently half of the events will be missed. Expanding on this example so that there are now three closely spaced events each spaced from the other by a time just slightly less than the MEPR, the first and third event will always be acquired and the second one always missed. These examples illustrate how the MEPR specification, as it relates to the PhotoniQ in *Particle* acquisition mode, essentially defines the minimum resolvable particle interarrival time. The SAER specification on the other hand defines a very different limitation — one that affects the average event throughput of the system. The following example explains the behavior of the PhotoniQ as the average event rate exceeds the SAER. Events and their associated triggers are supplied to the PhotoniQ at a fairly regular interval. The average event rate is much less than the SAER and no two events are spaced closer than the MEPR. In this situation, the system experiences no bottlenecks and thus all events are acquired. As the average event rate (and trigger rate) is increased, the PhotoniQ will continue to acquire all events up to the point when the average event rate exceeds the SAER. Beyond this point, the system will acquire events at the SAER and consequently will drop some events to meet this condition. Regardless of how high the trigger rate becomes, the PhotoniQ will continue to acquire events uniformly in time at the SAER.

PhotoniQ Data Acquisition Mode: Image

The PhotoniQ's *Image* acquisition mode is used for scanned imaging applications where priority is placed on acquiring every event (i.e. pixel). Here acquisition speed is characterized using the Maximum Trigger Rate (MTR) specification. Similar to the MEPR in that it specifies how closely two consecutive events can be spaced apart; the MTR is a specification that applies to sustained high event rates, whereas the MEPR is more appropriate for small bursts of events. For instance, provided that no two trigger signals are spaced closer than the MTR, a PhotoniQ in *Image* acquisition mode will acquire all triggered events up to the point at which its SDRAM buffer becomes full. After that point, events will be dropped in a somewhat unpredictable way. When the average trigger rate is low relative to the

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SAER, the PhotoniQ in *Image* acquisition mode behaves much like one in *Particle* acquisition mode — as long as the MTR specification is met, all events are acquired. Typical scanned imaging systems however, operate in a manner quite differently from this. A fixed rate pixel clock is supplied to the PhotoniQ as a trigger and runs for a period of time that allows the entire subject to be scanned. One event is generated for each pixel clock as long as the clock rate complies with the MTR requirement. In a high resolution application such as fluorescence confocal microscopy, the average event rate during the scanning period can far exceed the SAER specification for the PhotoniQ. After the scanning period has expired, the trigger is removed from the PhotoniQ and no more events are acquired. A PhotoniQ in *Image* acquisition mode handles this data *surge* by accumulating the fast, large *snapshot* of image data in its SDRAM buffer. If the SDRAM is sized such that it can hold an entire image, the accumulation of events is uninterrupted and all pixels are acquired. Throughout the acquisition period, the PhotoniQ outputs data to the PC while it is accumulating data into its buffer. Since the PhotoniQ's peak transfer rate to the PC is at the SAER specification, the PC transfer time can be much longer than the acquisition time, especially in high resolution imaging systems. It is not unusual to have a transfer to acquisition time ratio of five or ten to one. To prevent buffer overflow, the system must remain idle long enough so that the remainder of the buffered data can be transferred to the PC. The idle-to-active duty cycle must therefore be on the order of the transfer-to-acquisition time ratio.

Two examples of a PhotoniQ in *Image* acquisition mode are compared. In both cases the pixel clock rate is just within the MTR requirement and enough idle time is appropriated between image acquisitions so that the PhotoniQ can transfer the contents of its buffer to the PC and not overflow. The PhotoniQ's *Trigger End Count* parameter is set to match the number of pixels in each image, which is the same for both cases. The first example however utilizes an SDRAM buffer in the PhotoniQ equal to the image size, whereas in the second example it is half the size. As expected, the PhotoniQ in the first example counts up to the *Trigger End Count* while reliably acquiring all pixels. The whole image is transferred to the PC at a rate equal to the SAER where it is then completely reconstructed. In contrast, a large data loss occurs in the second example when the PhotoniQ's buffer becomes full and all subsequent triggers are rejected. The rejected triggers lead to a condition where the *Trigger Count* does not reach the *Trigger End Count* during the acquisition period. As a result, the PhotoniQ remains armed waiting for the missed triggers during the idle period which are then finally received at the start of the next acquisition period (image frame). The data transfer process to the PC occurs at the SAER and is initially unaffected by the missed triggers during acquisition. Eventually the transfer process makes up for the missed events by transferring events from the next image frame. The resulting reconstructed image ends up as a mix of data from two consecutive images. If acquisition is forced to continue, the situation becomes worse with more frames overlapping during image reconstruction. Buffer overflow of this type can be avoided by ensuring that the PhotoniQ's buffer size exceeds the image size. Although not described in the example, it is also important that the acquisition idle time be long enough so that the acquired image can be completely transferred to the PC. Similar overflow behavior will occur if this requirement is not met. While it is relatively easy to meet the conditions to prevent overflow, the PhotoniQ does offer a feature that allows overflow conditions to be detected should they occur. The event packets generated by the PhotoniQ can be configured to include either time stamps or trigger stamps. Time stamps are generally useful when operating the unit in *Particle* acquisition mode where each acquired particle can be tagged with a time of arrival. However, in *Image* acquisition mode, the trigger stamp is more appropriate because each acquired event (pixel) is tagged with the number of triggers seen by the system since the prior event was acquired. Ordinarily, the trigger stamp will increment by one with each pixel that is acquired. This indicates that no triggers, and therefore no pixels, were missed. When the trigger stamp increments by more than one (it can never increment by zero), the increment amount minus one indicates how many triggers were missed between acquired events. In an appropriately designed system, the trigger stamp serves as a meaningful diagnostic tool.

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PhotoniQ Data Acquisition Mode: Display Only

The *Display Only* mode is an invaluable acquisition mode that is most useful when it is essential to observe real-time event data such as when setting up and verifying a system for the first time. For systems that trigger at very slow rates, the *Display Only* mode is also quite effective because it operates like *Particle* mode but with a real-time data display. The acquisition behavior in *Display* mode (i.e. the means by which triggers are accepted and rejected) is identical to that of *Particle* mode except that the sustained average event rate is limited by the display overhead instead of the transfer rate to the PC. Where in the case of *Particle* mode this rate is specified as the SAER, the transfer rate in *Display* mode is limited by many factors including the PC's hardware and the types of applications it is running. Nonetheless, the sustained average event rate while in *Display* mode will often be less than 50 Hz.

PhotoniQ Data Acquisition Mode: Display & Log

Like the *Display Only* mode, the *Display & Log* acquisition mode passes data to the graphical user interface for real-time display. It also includes a logging capability that allows each displayed event to be logged to disk. Acquisition behavior is similar to the *Display* mode.

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