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Authors
Eid, JS
Muller, JD
Gratton, E

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Data acquisition card for fluctuation correlation spectroscopy allowing full access to the detected photon sequence

John S. Eid, a Joachim D. Müller, and Enrico Gratton
Laboratory for Fluorescence Dynamics, Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801-3080

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Typically, fluctuation correlation spectroscopy (FCS) data acquisition cards measure the number of photon events per time interval (i.e., bin)—time mode. Commercial FCS cards combine the bins through hardware in order to calculate the autocorrelation function. Such a design therefore does not yield the time resolved photon sequence, but only the autocorrelation of that sequence. A different acquisition method which measures the number of time intervals between photon events has been implemented—photon mode. This method takes advantage of the fact that in FCS the rate of photon counts is much less than the frequency of the clock that is used to determine the temporal location of the photons. By using this new mode of data acquisition, the current card design allows for 25 ns time resolution. The data acquisition card can operate in both time and photon mode and yields the time resolved sequence of photon arrivals in both cases. Therefore, the data is available for analysis by any method(s), such as but not limited to, autocorrelation, photon counting histogram, and higher order autocorrelation. © 2000 American Institute of Physics. [S0034-6748(00)05202-3]

I. INTRODUCTION

Fluctuation correlation spectroscopy (FCS) is a technique used to obtain kinetic information through analysis of the stochastic fluctuations in a molecule’s fluorescence.1 FCS obtains kinetic information by calculating the correlation in the emitted photon sequence. For example, since the excitation volume is known, the correlation resulting from the time it takes for a dye to cross this volume intrinsically yields a diffusion coefficient. In addition, any property which causes fluctuations in the fluorescence intensity of the dye at a rate fast enough to occur during the fraction of time when the dye is in the excitation volume, will be captured by the autocorrelation function. Therefore, such processes as rotational diffusion,2,3 triplet state formation,4 and chemical reactions5-9 can all be measured using this technique. For the most part, commercially available hardware correlators are used to perform the autocorrelation in FCS experiments. The electronics of the commercial correlator boards determine the autocorrelation function by a time resolved binning of the photon counts.10 The width of the time bin determines the temporal resolution of the autocorrelation function.

However, autocorrelation is not the only analysis that one can perform on the stream of photon counts. Since the determination of the autocorrelation function is a data reduction technique, some of the information embedded in the temporal sequence of the detected photons is lost. Other analysis techniques can yield additional information not contained in the autocorrelation. For example, photon counting histogram (PCH) analysis,11 higher order autocorrelation,12,13 and moment analysis14 have been introduced to analyze fluorescence fluctuation data. So any data acquisition method that only determines the autocorrelation, but does not return the complete time resolved sequence of photon events, neglects useful information. In order to avoid this limitation a data acquisition card which returns the entire time sequence of photon arrivals was developed.

II. CIRCUIT PHILOSOPHY

The card is designed so that it can acquire data in two different modes, which we call time and photon mode. Time mode determines the number of detected photons that occur within a fixed bin size determined by the frequency of the clock. Photon mode, on the other hand, determines the number of clock cycles between successive photon events. A schematic description of these two modes is shown in Fig. 1. In time mode the time lapse between two successive clocks determines the bin and the counts are given by the photon events. In photon mode the bin is determined by two successive photons and the clock yields the counts. In either mode the time resolution of the data is determined by the frequency of the clock. However, the data transfer rate to the host computer is mode dependent. Data is transferred at the frequency of the clock in time mode and at the frequency of the photon counts in photon mode.

If the clock frequency is greater than the average photon rate, then the data transfer efficiency of the photon mode is greater then for the time mode. This difference in the data transfer efficiency between the two modes is crucial because achieving a time resolution of 100 ns or better requires a clock frequency of 10 MHz or faster. Even with current computing speed [e.g., peripheral component interconnect (PCI) architecture], transferring data from a peripheral device through the computer’s bus at that frequency over an extended period of time, is not trivial. Commercial autocorrelators get around this problem by transferring the autocorrelation and not the raw data, thus limiting their applicability as
The overall experimental setup is shown in Fig. 2. A more detailed description is given by Berland et al.\textsuperscript{15} The laser used as the two photon excitation source is a mode-locked Ti:sapphire (Mira 900, Coherent, Palo Alto, CA) pumped by an Innova 410 argon ion laser (Coherent, Palo Alto, CA). The detection of emitted photons is through an inverted Zeiss Axiovert 135 TV microscope (Thornwood, NY) with a 63\texttimes\ Fluor oil immersion objective (numerical aperture=1.3). The one channel version of the card is a 16 bit industry standard architecture (ISA) board that has two inputs. The two inputs are the clock and the photon events. The photon events come from either a photomultiplier tube (Hamamatsu, R5600-04-P) or an avalanche photodiode (APD) photon counting unit (EG & G, SPCM-AQ-141). Since the APD unit outputs TTL pulses it is directly hooked up to the card. The photo multiplier tube (PMT) signal on the other hand first goes through an amplifier (Phillips Scientific: Model 6931, Ramsey, NJ) and is then passed through to a discriminator (Phillips Scientific: Model 6930) that outputs TTL pulses. The stored time sequence of photon events was analyzed by programs written for PV-Wave version 6.10 (Visual Numerics, Inc.) and with LFD Globals Unlimited software (Champaign, IL).

Rhodamine purchased from Molecular Probes (Eugene, OR) was dissolved in de-ionized water to a final concentration of approximately 3 nM. Then 500 \( \mu \)L from this dilution was placed on a hanging drop slide with a cover slip placed on top to make a watertight seal with the surface of the slide. The 20 base pair DNA duplex purchased from Midland (Midland, TX) was singly labeled with fluorescein and purified.\textsuperscript{16} It was then diluted to a concentration of 30 nM in buffer. The pH 8.4 buffer used consisted of 50 mM TrisCl, 200 mM NaCl, and 0.5 mM ethylenediaminetetraacetic acid. Then 0.8 M urea was added to the DNA-buffer solution. Approximately 500 \( \mu \)L of this sample was placed in one well of an eight chamber cover glass system (Nalge Nunc Inc., Naperville, IL).

**IV. OVERALL CIRCUIT SETUP**

The main design goal was to create a data acquisition board that preserved the entire time sequence of photon arrivals at a resolution of 100 ns or better. The detected photon sequence would then be transferred to computer memory and analyzed by software at some later point.

The overall structure of the data acquisition card can be seen in Fig. 3. There are four major components to the circuit. The first is the control logic that allows for switching between the two modes of the circuit as well as initialization of the card. The second component is the pulse generating logic. Two pulses are created causing a read of the counters’ output by the first in, first outs (FIFOs) followed by a reset of the counters. The third major component of the circuit consists of the four four bit counters which are incremented by the rising edge of the event signal during the temporal window determined by the timing signal. The last component is composed of two 4096 \( \times \) eight bit FIFOs and serves as a temporary storage buffer for the data before it is transferred by direct memory access (DMA) to computer memory.

The general sequence of operation for the data acquisition card begins with the detection of the timing signal. The counter is then blocked, preventing any more events from...
So usually one needs to trade off speed in order to reduce noise. Fortunately in going from the ACT type counters to the F counters the speed (max input frequency of 125 MHz) was unaffected while the noise was drastically reduced. Capacitors throughout the board are necessary in order for a wire-wrapped board to function at 60 MHz, and beyond. Low pass filters on the DMA request and acknowledge lines where required since our tri-state FIFOs are directly hooked up to the computer’s bus. If those filters are not employed then any noise on those lines could cause the card to hang up, or the computer to crash. Delay logic that prevents the card from making a DMA request too soon after the end of a DMA acknowledge turned out to be necessary in dealing with timing discrepancies between different computer motherboards. Different buses have different timing and noise constraints for the DMA transfer protocol even though the overall structure is standardized.

The DMA single transfer protocol is used to transfer the data from the FIFO to the bus. The empty flag of the FIFO is used as the DMA request signal and the DMA acknowledge pulse from the computer causes the FIFO to write to the bus (i.e., the DMA buffer). Software is then utilized to handle the transfer of data from the DMA buffer to permanent memory (i.e., hard drive, disk, etc.) and ensure that the user is notified if data that has not yet been transferred is overwritten in the DMA buffer. This software is also used to calculate the autocorrelation function and the average counts per second on the fly.

V. TIME MODE

In time mode the event signal is the output from the photon detector, while the timing signal is the output of a frequency generator (i.e., the clock). So the counters on the card will increment every time a signal is received from the detector. When the rising edge of the timing signal is received, the FIFOs read the number that the counters reached and (when the DMA request is acknowledged) write that information to the DMA buffer which is then taken to permanent memory. So the output is a string of numbers, each of which represent the number of photon events between successive rising edges of the clock. Since one cannot know where in the time bin a photon has arrived, the maximum time resolution will be determined by the width of the time bin. Therefore, the greater the clock frequency the higher the temporal resolution. In this mode the data acquisition card requests the bus at the clock frequency. For ISA single mode DMA transfer the maximum theoretical transfer speed is about 0.8 MB/s, which puts a theoretical upper bound on the order of 1 μs, on the time resolution of the time mode. Even if this frequency were attainable (we have found that a clock frequency of about ~0.5 MHz is the practical limit on most computers) this would mean that on average the FIFOs would be transferring zeros, since the photon count rate is an order of magnitude slower. A lot of bandwidth is therefore wasted since the bus transfers 16 bits every time it acknowledges the card’s request and yet only the first few of those bits would ever be used.

In order to insure proper operation in this mode the event
signal to the counters is disabled for the duration of the dead
time. This is done to prevent the counters’ output from
changing during the time in which the FIFOs are accessing
that output.

VI. PHOTON MODE

This mode was designed to overcome the data transfer
inefficiency inherent in the previous transfer protocol. In
photon mode the event signal is the clock and the timing
signal is the output of the single photon detector. When a
photon event is detected, the FIFOs read the output bits of
the counters and then the counters are reset. The counters
then count up every time the rising edge of a clock is de-
tected until the next photon event. So the output in photon
mode is a string of numbers each of which indicate the num-
ber of clock cycles between photon events. Again, the higher
the frequency of the clock the better one knows the temporal
resolution of each photon’s arrival. In contrast to the time
mode, however, the transfer rate is determined by the fre-
quency of photon events and is independent of the clock
frequency.

Although at first glance it may appear that the two
modes of operation (time and photon) are entirely symmet-
ric, this is in fact only the case when testing is done with
frequency generators. The moment the circuit is actually
tested with TTL pulses from a photon detector setup the
symmetry is broken because whether or not the timing signal
is periodic (time mode) or random (photon mode), matters.
The circuity required for the photon mode is more complex
because certain safeguards need to be put in place to handle
the random timing signal. One such safeguard involves dis-
abling the timing input line between the time that a stop
signal arrives and the time the circuit is once again ready to
receive counts (i.e., during the dead time). This is necessary
because in this mode it is possible for another timing signal
(i.e., photon) to arrive during the dead time of the card. In
time mode on the other hand we needed to stop only the
event signal’s input line. In fact, blocking the timing signal’s
input line in time mode will result in the creation of extra
rising edges due to the 50% duty cycle nature of the output
of frequency generators. So the circuit was designed to rec-
ognize its mode of operation and block the timing event line
only in the photon mode for the duration of its dead time of
25 ns.

Other safeguards revolve around the fact that in the pho-
ton mode the circuit needs to be able to handle a large dy-
namic range in the separation between two consecutive tim-
ing signals. This is a result of the uncertainty in the time
lapse between any two consecutive photons. In the case of a
burst of photons it is important to have a FIFO that has
enough depth such that it will not overflow while it waits for
the computer to respond to its DMA request. On the other
hand, if there is a long time separation between two photons
and the frequency of the clock is high then it becomes pos-
sible that the counters could overflow. The lower the average
rate of photon counts the more likely a scenario this be-
comes, since the average time between successive photon
events increases. A relationship between the probability of
having a counter overflow and the average photon count rate
can be derived, assuming that the photon detection process
can be modeled as a homogenous Poisson point process.

\[ P(t) = ke^{-kt}, \]

where \( k \) is the average number of photons detected per
second.\(^{20}\) We define an overflow time, \( t_{of} \), such that succes-
sive photons separated by a time greater than \( t_{of} \) cause the
counters to overflow. To calculate the probability, \( P(overflow) \),
that the two photons are separated by at least the overflow
time, \( t_{of} \), one needs to integrate Eq. (1)

\[ P(overflow) = 1 - \int_{0}^{t_{of}} P(t) dt = e^{-kt_{of}}. \]

The overflow time, \( t_{of} \), depends on the frequency of the
clock, \( f_{\text{clock}} \), and the number of bits, \( n \), of the counter in the
following manner:

\[ t_{of} = \frac{2^n}{f_{\text{clock}}}. \]

Equation (2) is the probability of an overflow condition oc-
curring given a single pair of photon events. In order to take
into account the total number of photons, \( M \), received in an
experiment, we need to modify Eq. (2)

\[ P_{M}(\text{overflow}) = 1 - (1 - e^{-kt_{of}})^M. \]

From Eq. (4) we have the probability of a single overflow
occurring given \( M \) photons, \( P_{M}(\text{overflow}) \), as a function of the
average number of photons detected, \( k \), for a constant
overflow time, \( t_{of} \), determined by the card design. This func-
tion transitions from 1 to 0 over a small range of the variable
\( k \). The range over which this transition occurs depends on
the number of bits, \( n \), the clock frequency, \( f_{\text{clock}} \), and the num-
ber of photons received, \( M \). So for example, if \( n, f_{\text{clock}} \), and
\( M \) are set to 16 bits, 10 MHz, and ten million photons, re-
spectively, then the range of \( k \) in which \( P_{M}(\text{overflow}) \) tran-
sitions from 0.97 to 0.03 is 2268–2992 cps. As \( k \) is increased
above 3000 cps the probability of a counter overflow quickly
becomes vanishingly small.

As an additional safeguard we disable the counters once
their maximum count is reached. This is done to insure that
if there is an overflow the counters will not turn over and
begin counting from zero again. So the output of the counters
would be 2\(^{10} \) regardless of the magnitude of an overflow.
Due to this design, counter overflows can easily be spotted in
our saved data and dealt with as desired. In the interest of
future card design the earlier equations can be used to calcu-
late the number of bits, that a counter should possess, such
that the probability of an overflow is negligibly small, even
for a worst case experimental scenario. In other words for a
case in which a high time resolution is required and yet a low
flux of photons is available. If we assume a clock frequency,
\( f_{\text{clock}} \), of 100 MHz, an average number of photons, \( k \), of 200
cps and the detection of ten million photons, \( M \), then the probability of an overflow, \( P_{M}(\text{overflow}) \), would be about
\( 3 \times 10^{-8} \) if the counter had 24 bits.
the autocorrelation function that was calculated through soft-
the presence of a photon in that bin
ns. The data were taken in photon mode and Fig. 4
nM rhodamine using an APD with a time resolution of 100
three spikes that repeat every 20
photon mode output alternated between two numbers
fi
one, both, and then one pulse. The separation between the
and the other 3 being ones
double pulse generator. In time mode this would have been
number of clocks between each pulse arriving from the
three spikes had a ratio of 1:2:1 re
fi
2
fi
VII. RESULTS
Systematic tests using frequency generators were per-
formed to test our electronic circuit and software design. One
of the tests for the photon mode involved a double pulse
generator as the photon input and a 9 MHz clock input. The
two pulses were separated by 200 ns and repeated every 20
µs. Performing an autocorrelation on the data resulted in
three spikes that repeat every 20 µs. The magnitude of the
three spikes had a ratio of 1:2:1 reflecting the overlaps of
one, both, and then one pulse. The separation between the
first and second spike was the same as the separation be-
between the second and third spike, and equal to 200 ns. The
photon mode output alternated between two numbers (178
and 2) repeated over and over again. These signified the
number of clocks between each pulse arriving from the
double pulse generator. In time mode this would have been
stretched out to 181 numbers with 178 of them being zeros
and the other 3 being ones (representing either the absence
or presence of a photon in that bin).
In a subsequent experiment we measured a solution of 3
nM rhodamine using an APD with a time resolution of 100
ns. The data were taken in photon mode and Fig. 4(a) depicts
the autocorrelation function that was calculated through soft-
ware. The autocorrelation, $G(\tau)$, decays to zero, indicating
that the fluctuations in intensity are independent over long
time scales. The correlation time due to the kinetic process of
diffusion is seen to be on the time scale of $\sim$50 µs. The fit
with a simple translational diffusion model, assuming a 3D-
Gaussian for the two-photon excitation volume, is excellent,
except for the deviation on the 100 ns time scale due to a
very strong correlation. Figure 4(b) demonstrates that this
correlation is due to afterpulsing of the APD, since it persists
even for a scattered laser light experiment in which the pho-
ton counts are not correlated on the time scales accessible by
our experimental setup.

In the experiments mentioned earlier we have not uti-
лизed the fact that our data acquisition card preserves the
entire time resolved sequence of photon arrivals. One major
advantage to having all of the data is that it is possible to use
analysis other than the autocorrelation function. For ex-
ample, one can utilize the fact that the histogram of photon
arrivals from a fluctuating intensity source is broader then
the Poissonian expected of a constant intensity source as can
be seen in Fig. 5. For a single species the two parameters that
can be used to fit this super-Poissonian curve are the bright-
ess per molecule, $\epsilon$, and the number of molecules within the
observation volume, $N$. For the general case of $m$ species,
$2m$ parameters are required to fit the curve. By using this
analysis one can determine the number of species present in
the sample. In this case “species” is defined as a mole-
cule of a specific brightness. Therefore, this method can be
used to distinguish between particles that have different
brightness but similar diffusion coefficients. In many bio-
logical applications, such as the study of dimer formation
and dissociation, or enzyme/substrate interactions, the

![Rhodamine 110 at 3 nM](Image)

**FIG. 4.** Photon mode autocorrelation data taken with a 10 MHz clock fre-
quence. (a) Rhodamine data (courtesy of Dr. Yan Chen) depicting APD
afterpulsing correlation on the order of 50 ns. The correlation due to the
diffusion of Rhodamine through the excitation volume is on the order of 50
µs. The autocorrelation decays to zero indicating no correlation for long
time scales. (b) Autocorrelation of scattered laser light data which correbo-
rates the premise that the 50 ns correlation is due to detector afterpulsing
and not due to fast kinetics. Without afterpulsing the scatter data would not
exhibit any correlation on the time scales measured.

![PCH Analysis of Raw Data](Image)

**FIG. 5.** PCH analysis of data obtained from free fluorescein in solution. The
deviation from the predicted Poissonian becomes more pronounced towards
the tail. The data can be fit using two parameters: the average number of
molecules in the excitation volume, $N$, and the number of counts per second
per molecule (i.e., the brightness), $\epsilon$. The residuals of this fit are also shown
indicating a good fit of the data. This analysis complements that of the
standard autocorrelation because it can separate species based on differences
in brightness as opposed to differences in the diffusion coefficient. The
emphasis here is on the flexibility in analysis due to the fact that the entire
temporal sequence of photon arrivals is stored.
change in the diffusion coefficient is too small to be measured using the autocorrelation function. On the other hand, if the components of the reaction are singly labeled then the brightness will change by a factor of two upon association, and the interaction kinetics can be observed using PCH.

Another advantage resulting from preserving all of the data is that any artifacts present in the original data can be examined and removed if necessary. In Fig. 6(a) an example is shown where a large flux of photons occurs for a short time during the experiment. Figure 6(b) shows that a skewed curve results when the autocorrelation of this data is calculated. The cycle, which represents a full DMA buffer transfer (32 Kb), where the anomalous photon flux occurred can be located and edited out. The resulting autocorrelation, seen in Fig. 6(c), can now be used to calculate a diffusion coefficient. Hardware autocorrelators can only yield the curve shown in Fig. 6(b). This leaves the experimentalist with the unpleasant choice of either retaking the data or attempting to backtrack from the skewed autocorrelation curve by some complex fitting routine with no guarantee of success in either case. In the case where the anomalies themselves may be of interest the task of studying them without interference from the bulk of the data is only feasible if it is possible to directly work with the original data set. One can also imagine running an experiment where the conditions are changed at specific times during the data acquisition process. It is much easier to analyze the different parts of that experiment separately as opposed to trying to make heads or tails of the convolution resulting from the autocorrelation function of the total data set. The earlier examples are by no means an exhaustive list but are intended to give a flavor of the variety of options that are now accessible with this data acquisition card.

VIII. DISCUSSION

Different approaches have been taken over the years to produce faster correlators. The need for correlators sprang up once the field of photon correlation spectroscopy (PCS) began in the 1970’s. During that time computers were slow and expensive so the result was the production of no-memory hardware correlators. The hardware design improved from single channel delayed-coincidence measurements, to single stop time-to-amplitude conversion techniques, and then to multistop systems. Nowadays there are excellent multiple-tau hardware correlators available, in terms of resolution and functionality. However, these hardware correlators have two main disadvantages: price and data analysis flexibility. The price is high due to the electronics required to perform the correlations in hardware as well as the need for high temporal resolution. Data analysis flexibility is lost because hardware correlators only return the autocorrelation function and not the raw data.

The incredible increase in computing power has made analysis through software not only feasible, but in most cases more convenient then using highly complex, and therefore expensive, hardware. To analyze the raw data produced by our digital card, we developed software that performs the calculation of the autocorrelation in linear and log time scales as well as in real time. One can therefore use multiple analysis methods on the same data set as well as edit the raw data itself thus giving a more complete picture of the experiment, as was shown in Sec. VII.

Our card reaches the time scale of most hardware correlators and preserves all the information about the temporal distribution of the photons. It also has better dynamic range then commercial hardware correlators since the correlation

FIG. 6. Time mode data of 20 mer DNA singly labeled with fluorescein taken with a 10 kHz clock frequency. (a) Average counts per bin (0.1 ms) averaged over each DMA buffer transfer cycle (32 Kb) showing an abnormal flux of detected photons in one cycle out of the 250. (b) Autocorrelation of the raw data. (c) Autocorrelation of edited data. The data was edited by removing the cycle in which the abnormal flux of detected photons occurred. This is only possible because the full data set is saved in memory, not just the autocorrelation.
can go all the way out to the entire time span of the experiment.

For a given clock frequency the information content of the time and photon modes of our card is, for the most part, equivalent. Deciding which mode to use involves comparing the average number of photons detected per second with the maximum transfer rate of the data acquisition card. One would have to use the time mode if the photon flux rate exceeded the transfer rate of the card (approximately 500 kHz for an ISA card and 10 MHz for a PCI card). To determine which mode has a greater data transfer efficiency one need only compare the frequency of the clock to the average counts per second expected from the sample. The photon mode is more efficient when the average counts per second is less than the clock frequency and vice versa for the time mode. Typically, since we are operating in the single molecule regime for FCS measurements, the average counts per second is less than 100 000. The clock on the other hand is set to as high a frequency as possible due to the need for greater time resolution. In such a scenario, the photon mode is several orders of magnitude more efficient, in terms of data transfer rate, than the time mode.

The design discussed in this article is an ISA data acquisition card. For a PCI version of the card it would be more advantageous to use the PCI bus master transfer protocol which would allow for 32 bit data transfer at a sustainable clock speed of about 10 MHz. One might imagine that with this kind of transfer speed the data transfer efficiency of the photon mode is no longer needed. However, wasting bandwidth is never a good option. The extra speed now available due to advances in computing technology can be used for other things, such as transferring more channels, performing additional real-time calculations on the incoming data, etc.

Since the bus transfer is done with either 16 or 32 bits at a time it is inefficient to perform data acquisition in such a way that only the first couple of bits are ever nonzero. In other words, time mode would be a viable option only if it were possible to transfer one or two bits at a proportionally higher rate then it is to transfer 16 or 32 bits. But even if that were possible, the photon mode would still have an advantage because it is a more compact representation of the same information, thus reducing the amount of memory needed to store the data. For a photon rate of 100 kHz, a clock frequency of 10 MHz, and 1000 s of data collection the file will be on the order of 100 Mb for the photon mode and 10 Gb for the time mode.

Ideally one would like to have the counters enabled the entire time because the ultimate limitation on the temporal resolution of this data acquisition card is its finite dead time—the time during which the counters are disabled. This is not possible because some time must be taken, after every timing event is received, to transfer the data to the FIFOs and reset the counters. For our current design, the time during which the counters are disabled is 25 ns. On the other hand, the APDs we used have a dead time of around 30 ns due to their internal quenching circuitry. The PMT we used in our experiments had a dead time on the order of a couple of nanoseconds but its afterpulsing obscures all processes that occur on the microsecond and shorter time scales (data not shown). Therefore, the current limit in time resolution is due to detector effects and not the card’s dead time. A scheme involving two entirely independent channels receiving counts from two independent detectors has been used to get around these limitations. This design consists of continuously alternating between the two independent channels either through hardware or software. In this manner, first order effects due to afterpulsing and dead times would be eliminated since neither of those effects would correlate across the independent channels. This design would require the use of a beam splitter to separate the signal onto the two detectors, thus resulting in the loss of half of the photon counts. Higher order dead time effects will still be present since it is possible to have more photon events during the dead time, then independent channels. But these effects would, for the most part, be negligible. It is worthy to note that the general structure of our single channel data acquisition board is such that it can easily be scaled to multichannel capability. So, in order to construct a multichannel version one simply needs to place multiple copies of the current version on the same board, in addition to some very simple logic that continually switched between the channels after each photon is received. This is of great interest because a multichannel version of this board will allow for the use of such techniques as fluorescence resonance energy transfer and cross correlation.

IX. CURRENT DEVELOPMENTS

In collaboration with ISS (Champaign, IL) a dual channel, 32 bit, PCI version of our design has been implemented. Unlike the switching scheme proposed earlier, this card saves all of the data from both channels. In this way one can do the switching through software at some later time, to nullify dead time and detector artifacts, or analyze each of the channels independently, since none of the photons are lost. The cost of using this method is that it transfers data at half the speed and takes up twice as much memory as the hardware switching scheme mentioned earlier.

In addition to a multichannel version of the data acquisition card, other functions can be developed that would provide additional information. The raw data, from either mode of operation, gives information about the temporal separation between photon arrivals but does not yield the time lapse between laser excitation and fluorescence emission—the lifetime. Combining this extra dimension to the available FCS analysis yields a very powerful observational technique. To address this issue we have designed additional circuitry that is used to measure the lifetime of the detected fluorescence. In conjunction with a multichannel version of the card this addition will allow us to obtain multiple order autocorrelation functions, PCH, cross-correlation function, and lifetime information from a single experimental data set.

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18. For each individual computer the maximum practical card transfer speed in time mode can be experimentally verified by noting the frequency of the clock at which the DMA acknowledge signal just begins to overlap with the DMA request signal.
19. Strictly speaking the photon detection process is a doubly stochastic Poisson point process (see Ref. 20), but it is well approximated by a homogeneous Poisson point process for the relatively long time scales of interest here.