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Goro Nishimura

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Goro Nishimura
Research Institute for Electronic Science, Hokkaido University, Sapporo 001-0020, Japan

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A photon timing recorder was realized in a field programmable gate array to capture all timing data of photons on multiple channels with down to a 1-ns resolution and to transfer all data to a host computer in real-time through universal serial bus with more than 10 M events/s transfer rate. The main concept is that photon time series can be regarded as a serial communication data stream. This recorder was successfully applied for simultaneous measurements of fluorescence fluctuation and lifetime of near-infrared dyes in solution. This design is not only limited to the fluorescence fluctuation measurement but also applicable to any kind of photon counting experiments in a nanosecond time range because of the simple and easily modifiable design. © 2015 AIP Publishing LLC.

Fluorescence fluctuation spectroscopy (FFS) is one of the most sensitive analytical methods for fluorescent particles at the single molecule level. Among this, the correlation analysis of the fluorescence fluctuation of fluorescent particles in solutions is so-called fluorescence correlation spectroscopy (FCS) and now widely known as a very useful tool in chemistry, biophysics, cell biology, and medicine. The key in FCS is a single photon counting technique with high efficient detection of the fluorescence from single molecules achieved by a confocal optics or evanescent field, which makes a very low background detection. Once the single photon is detected with such optics, which specifies the polarization, wavelength, and position of detection, the arrival time of the photon is the only remained information carried. Therefore, the lossless detection of the photon timing with a high timing-resolution is the key issue. In this note, a very simple design of the time recording with a high-speed serial communication logic in a field programmable gate array (FPGA) is demonstrated in the fluorescence fluctuation analysis with down to a 1-ns resolution. Similar works to construct a digital correlator in FPGA with 4-ns resolution, and more recently, a 32-channel FPGA based correlator with photon counting module has been published. Further, a digital frequency domain (DFD) fluorescence lifetime imaging (FLIM) system designed on a FPGA has been reported. This was also a photon counting system but embedded a specific circuit for frequency domain measurements. Our design avoids such cumbersome design for specific purpose but takes a more flexible approach, transferring all timing information to a host computer with universal serial bus (USB). In this note, we present the keys of design, how the data are captured and transferred by USB2.0, and demonstrate some applications.

The key concept of this design is that a single photon counting time series is regarded as a serial digital communication data. The transfer rate of the serial communication technology nowadays is beyond gigabits per second and this rate seems to be sufficiently fast to capture the photon timing without loss with a nanosecond timing accuracy because the count rate in almost all photon counting measurements is far below $10^9$ counts/s. Further, a nano-second time resolution is sometimes sufficient to extract fluorescence lifetime in case of fluorescence measurements. To realize this idea, we employed a gigabit serial interface logic in FPGA, called SERDES (serializer/deserializer) to capture the bit streams of detected events. This logic reduces a serial gigabit stream to an order of 100 MHz parallel data to manage the further processing in the FPGA. Some FPGAs have several SERDES logics in a single FPGA chip, which work individually with a master sampling clock. Then, all the bit stream data captured by input SERDES (ISERDES) are transferred to a host computer. We employed the USB2.0 interface for data transfer, which is the most common and convenient interface for peripherals. The theoretical maximum transfer rate of USB2.0 is 480 Mbps. If the data size is assumed two bytes for each event, the transfer rate of event will be in an order of 10 M events/s. The average count rate of the photon detection is usually less than $10^6$ counts/s in photon correlation measurements, and therefore, the maximum number of input channels will be around few channels with consideration of the margin of transfer rate. In our design, we chose 2 or 4 input channels to fit the two bytes data size.

The actual device was constructed on a FPGA board with a daughter board (Papilio Pro, Gadget Factory). Details about the implementation can be found in section 1 of the supplementary material. The center large box in the diagram, as shown in Fig. 1(a), indicates an actual circuit constructed in a FPGA (XC6SLX9-2C, Xilinx). The sampling clock was fixed to 960 MHz, which is slightly below the maximum frequency, and four 4-bit ISERDES logics were embedded, reducing the processing clock at 240 MHz to satisfy the device specifications. The four individual inputs of ISERDES accepted low voltage transistor-transistor logic (LVTTL) signals from detectors or a reference through logic level translators on the daughter board. As a result, the input stream was sampled at
960 MHz and stored in a 4-bit register for four successive samplings. The latest sampling bit of the 4-bit register was kept in another 1-bit register for the edge detection. The event arriving time was detected from the transition of logic level. This makes a dead time with one successive sampling period. The slow time stamp was also kept in an 8-bit counter running at 240 MHz synchronized to the ISERDES timing in order to know the absolute time.

Then, the events in 4-sampling period were encoded in 2-bit data for coarse 2.083 ns resolution or 4-bit data for fine 1.042 ns resolution. Since there is a possibility of multiple events in the 4-sampling period, 4-bits are needed to keep all events with the fine resolution. Therefore, the total data for multiple inputs become the multiple of 4-bits or 2-bits by the number of channels with the fine or coarse resolution, respectively. The raw encoded data format for the data transfer is shown in Fig. 1(b). The events in the 4-sampling time were encoded in a 16-bit data, which consisted of the 8-bit detection bits and the 8-bit slow time stamp. The maximum average event is limited by this data size and the transfer rate of USB2.0. If more channels are needed with same time-resolution, more data bytes are required because transfer data are the byte unit. Therefore, the extension of the input channels makes a smaller acceptable maximum photon count rate.

Since USB is not a real time transfer technology, a buffer for transfer data is needed to eliminate loss of data. In our design, the depth of the first-in first-out (FIFO) memory was 8192. The maximum allowance of input was tested using periodic inputs from oscillators and about 18 MHz could be transferred without loss of data with the bulk transfer mode of USB using a USB transceiver (FT232H, Future Technology Devices International Inc.) and a simple receiving program on a Linux computer. As a result, we could estimate the maximum input rate about 4,5 \times 10^6 (counts/s)/channel with 2.083 ns resolution and 9.0 \times 10^6 (counts/s)/channel for 2-channel input with 1.042 ns resolution, respectively. It is worth to note that this value is average event rate. In the photon correlation measurements, the photons are bunched and the instantaneous count rate becomes higher than the average value. The FIFO can keep 8192 events and will overflow more than 8192 events within the minimum transfer time about 0.8 ms. The transfer rate and the FIFO depth here were sufficient for common experimental conditions of FFS less than 1 \times 10^6 (events/s)/channel and therefore all timing data could be recorded with negligible loss except the measurements with very large photon burst. Since the 8-bit counter for the time stamp was not sufficiently wide, zero time data were always transferred at the rollover of the counter to get the absolute time in the post process. Data transferred in the memory were analyzed on the host computer. The raw data were usually converted to a more efficient format, which consist of a 32-bits absolute time stamp with the fine or coarse time resolution and an 8-bits event data. The four unused bits remain for a future extension of input channels. This format reduces the data size when the event rate is not higher than about 200 kcps. The peripheral circuit (level translators and USB transceiver) was constructed on a universal circuit board as a daughter board of the FPGA board as shown in Fig. 1(c).

We have conducted a dynamic light scattering (DLS) measurement and a fluorescence fluctuation measurement of solutions with a continuous wave (CW) excitation source. First, we conducted DLS measurements to check this timing recorder and the post-process algorithm to calculate correlation function (CF) using latex microsphere solutions. The CFs were decaying exponentially and the correlation times were in very good agreement with the theoretical values from the size of the microspheres (details in section 2 of the supplementary material). Then, two fluorescence probe solutions, IR806-BSA (bovine serum albumin) complex and BSA coated water-soluble Qdots (CdSeTe/CdS) solutions, were tested in near-infrared FFS measurements with a homemade apparatus described in section 3 of the supplementary material. Then, two fluorescence probe solutions, IR806-BSA (bovine serum albumin) complex and BSA coated water-soluble Qdots (CdSeTe/CdS) solutions, were tested in near-infrared FFS measurements with a homemade apparatus described in section 3 of the supplementary material. Then, two fluorescence probe solutions, IR806-BSA (bovine serum albumin) complex and BSA coated water-soluble Qdots (CdSeTe/CdS) solutions, were tested in near-infrared FFS measurements with a homemade apparatus described in section 3 of the supplementary material. Then, two fluorescence probe solutions, IR806-BSA (bovine serum albumin) complex and BSA coated water-soluble Qdots (CdSeTe/CdS) solutions, were tested in near-infrared FFS measurements with a homemade apparatus described in section 3 of the supplementary material.

Finally, we conducted a simultaneous measurement of the fluorescence lifetime and fluorescence fluctuation of IR806-BSA and indocyanine green (ICG)-Intralipid solutions. A single photon photomultiplier tube module (H8631-50, Hamamatsu Corporation) and the post-process algorithm to calculate correlation measurements, the photons are bunched and the instantaneous count rate becomes higher than the average value. The FIFO can keep 8192 events and will overflow more than 8192 events within the minimum transfer time about 0.8 ms. The transfer rate and the FIFO depth here were sufficient for common experimental conditions of FFS less than 1 \times 10^6 (events/s)/channel and therefore all timing data could be recorded with negligible loss except the measurements with very large photon burst. Since the 8-bit counter for the time stamp was not sufficiently wide, zero time data were always transferred at the rollover of the counter to get the absolute time in the post process. Data transferred in the memory were analyzed on the host computer. The raw data were usually converted to a more efficient format, which consist of a 32-bits absolute time stamp with the fine or coarse time resolution and an 8-bits event data. The four unused bits remain for a future extension of input channels. This format reduces the data size when the event rate is not higher than about 200 kcps. The peripheral circuit (level translators and USB transceiver) was constructed on a universal circuit board as a daughter board of the FPGA board as shown in Fig. 1(c).
FIG. 2. Fluorescence correlation functions and fluorescence decay functions of IR806-BSA and ICG-Intralipid solutions.

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6See supplementary material at http://dx.doi.org/10.1063/1.493336 for detail descriptions on the FPGA design, DLS, and FFS experiments.