8.3 A Microsystem for Time-Resolved Fluorescence Analysis using CMOS Single-Photon Avalanche Diodes and Micro-LEDs

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Although microfluidics and microarray technologies are revolutionizing the throughput, sensitivity and cost in many areas of biodiagnostics [1], they are still reliant on bulky and expensive fluorescence analysis instrumentation. Conventional fluorescence intensity measurements are prone to misinterpretation due to illumination and fluorophore concentration non-uniformities. Thus, there is a growing interest in time-resolved fluorescence detection, whereby the characteristic fluorescence decay time-constant (or lifetime) in response to an impulse excitation source is measured.

We describe the first complete micro-scale time-resolved fluorescence analyzer to include excitation, detection and time-gated histogram generation (Fig. 8.3.1). It comprises a CMOS chip in 0.35μm high-voltage technology incorporating a 16×4 array of SPADs [2], digital counters and LED drivers bump-bonded to an AlInGaN UV micro-pixellated light-emitting diode (micro-LEDs) array [3]. This system replaces instrumentation based on lasers, photomultiplier tubes, bulk optics and discrete electronics with a PC-based micro-system.

The micro-LEDs in the 16×4 array are individually addressable, have a 100μm pitch and each have a diameter of 72μm. They were fabricated from 'standard' UV LED wafers grown on c-plane sapphire substrates by metal-organic chemical vapour deposition (peak emission 370nm). Electrical connection between the micro-LED array and the driving CMOS chip was made using a bump-bonding process with each element of the LED array being bonded to a dedicated driver site. Driving circuitry for each electrode is contained within a 200×100μm² cell, with 19090μm² of top metal exposed as the electrode surface. The array of electrodes had their passivation selectively removed at the foundry to reveal the aluminium top-plate electrodes for bump-bonding. A post-processing step of oxygen plasma ashing removed the polyimide layer on the chip, which has been shown to improve the photon detection probability of the SPADs by a factor of 2 to 5 [3].

The SPAD allows single photon detection through the action of avalanche breakdown in a p+/deep n-tub photodiode, reverse biased above its breakdown voltage (Geiger mode). The detection of a photon and the subsequent avalanche breakdown of the SPAD generates 40ns digital pulses with 114ps full width at half-maximum jitter, which are processed by time-gated on-chip ripple counters [4]. These counters are situated in a second array (Fig. 8.3.1) pitch-matched to the micro-LEDs, allowing histogram and lifetime analysis without the need for external photon counting hardware and significantly reducing the amount of data to be broadcast off-chip. Direct observation of SPAD output pulses is also possible from an array of addressable SPADs situated directly within the micro-LEDs for confirmation of the integrated lifetime analysis techniques. Figure 8.3.2 illustrates the optical paths of excitation light and the returning fluorescence emission to these SPADs from a sample held in a micro-cavity slide.

The counter array circuitry consists of X- and Y-address decoders, timing generation circuitry and the pixel array (Fig. 8.3.3). Each SPAD has two associated 9b ripple counters (Fig. 8.3.4) as well as time gate and address decode logic. The two counters allow photons from two different time periods to be collected simultaneously. This operation implements the 2-gate rapid lifetime determination (RLD) algorithm proposed in [5];

$$\tau = \Delta T / \ln\left(\frac{TG1}{TG2}\right) \quad (1)$$

where τ is the lifetime of the fluorophore, ΔT is the width of both of the time gates and TG1 and TG2 are the number of counts gathered in the first and second time gates, respectively.

SPAD pulses provide the asynchronous clock to the first T-type flip-flop (FF) in the counter. A ripple counter was chosen to minimize the clock loading, since no synchronous count behaviour is required. Time-gated operation is accomplished by providing the toggle input of the first T-type FF in the counter with short pulses, which are generated within the pixel from delayed versions of the 3.68MHz system clock broadcast to the array from the on-chip timing generator. Thus, the loading on the clock waveforms and the required bandwidth of the bus drivers are minimized.

The timing generator consists of a 120-element tapped delay line composed of current limited buffers. The buffer unit delay is 408ps with 44ps RMS jitter at 3.3V at room temperature. Three delayed versions of the 3.68MHz system clock are generated, each delayed output can be selected independently under the control of a latched shift register. Time-gate widths can be selected from 408ps to 48ns with a resolution of 408ps.

LEDs are addressed using the same X- and Y-decoders and electrical pulses are defined using the on-chip timing generator (Fig. 8.3.3). The drive circuitry associated with each micro-LED pixel is shown in Fig. 8.3.5. Short electrical pulses are applied to the micro-LED, with a width equal to the delay between SQIN and SQIND. The turn-on voltage of the UV micro-LEDs is around 4.5V, so high voltage D-MOS transistors have been employed in the driver circuit. Current pulses of 4.5mA at 13V are generated at a frequency defined by the system clock.

Photon counts due to directly coupled light from the LEDs have been minimized by choosing a 370nm UV excitation source where the photon detection probability (PDP) of the SPADs is 7%. This wavelength is also ideal for the excitation of quantum dot fluorophores. At 526nm, the emission wavelength of the Adirondack Green quantum dots, the PDP of the SPADs increases to 25%. Measurements were obtained using CdSe/ZnS quantum dots [6]. The quoted lifetime of these samples is 15 to 20ns.

Using the on-chip 2-gate RLD method with 10.2ns gate widths, lifetimes of 16.576ns, 17.349ns and 19.174ns for Adirondack Green (concentration: 77.03 nmol/ml), Catskills Green (68.77 nmol/ml) and Hops Yellow (57.17 nmol/ml) quantum dots were calculated, respectively. These are in good agreement with the lifetimes quoted by the manufacturer and those obtained by conventional measurement. Using SPADs situated within the micro-LED array, the decay of Adirondack Green and the instrument response function (IRF) were captured and the data processed by external Becker and Hickl SPC-730, photon counting hardware (Fig. 8.3.6). Using the on-chip time-gated counters, an equivalent fluorescence decay histogram of the Catskills Green quantum dot sample was captured in 26 time gates, each 800ps wide (Fig. 8.3.7).

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References:
[1] T. Vo-Dinh and M. Askari, "Micro Arrays and Biochips: Applications and Potential in Genomics and Proteomics," *Journal of Current Genomics*, vol. 2, No. 2, pp. 399-415, 2001.
[2] C. Niclass, M. Sergio and E. Charbon, "A Single Photon Avalanche Diode Array Fabricated in Deep-Submicron CMOS Technology," *IEEE Proc. of Design, Automation and Test in Europe*, vol. 1, pp. 81-86, March 2006.
[3] C. W. Jeon, H. W. Choi, E. Gu and M. D. Dawson, "High-density matrix addressable AlInGaN-Based 368nm microarray light-emitting diodes," *IEEE Photonics Technology Letters*, vol. 16, pp. 2421-23, 2004.
[4] D. Mosconi, D. Stoppa, L. Pancheri, L. Gonzo and A. Simoni, "CMOS single photon avalanche diode array for time-resolved fluorescence detection," *IEEE Proc. of ESSCIRC*, pp. 564-567, September 2006.
[5] H. C. Gerritsen, M. Asselbergs, A. V. Agronskaia and W. Van Sark, "Fluorescence lifetime imaging in scanning microscopes: acquisition speed, photon economy and lifetime resolution", *Journal of Microscopy*, Vol. 206, pt 3, pp. 218–224, June 2002.
[6] Evident Technologies, "EviDot Specifications", Date Visited: 29th November 2007, <www.evidenttech.com/products/evidots/evidot-specifications.html>

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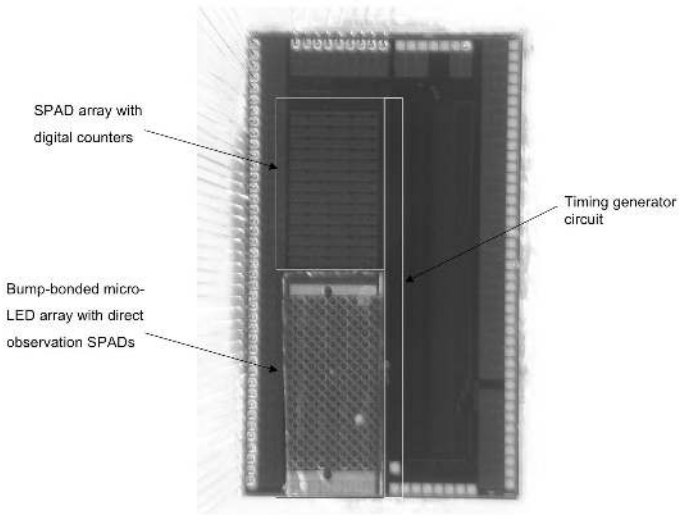

Figure 8.3.1: Chip Micrograph.

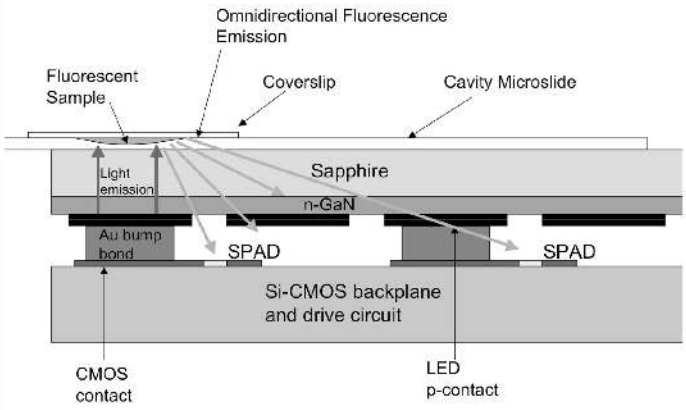

Figure 8.3.2: CMOS back-plane with bump-bonded micro-LEDs.

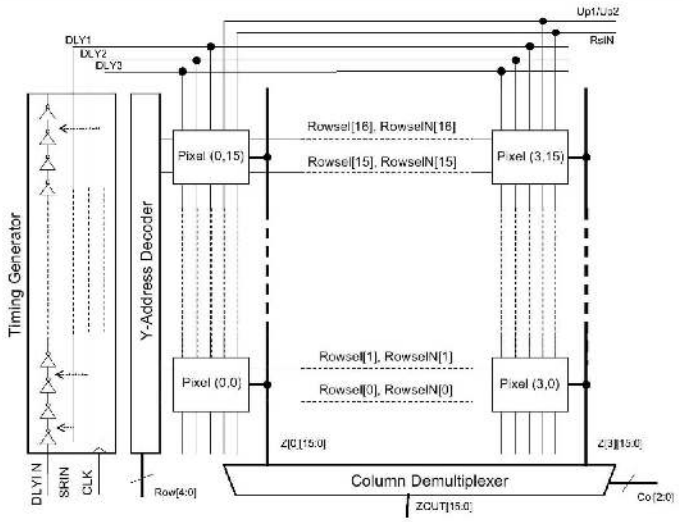

Figure 8.3.3: System block diagram. Both the micro-LED driver array and SPAD counter array are based on this architecture.

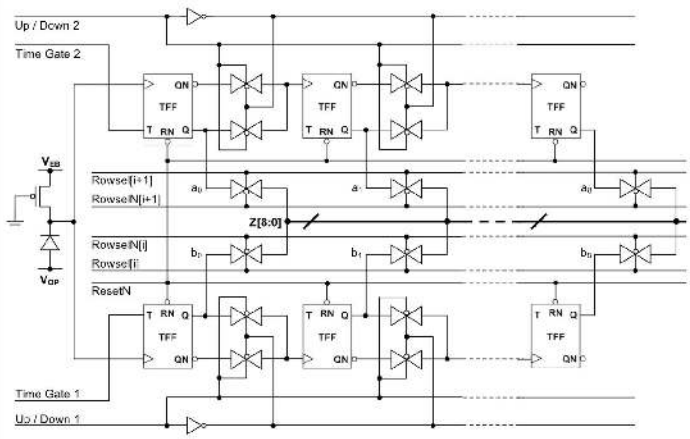

Figure 8.3.4: SPAD circuit with passive quench transistor and two associated 9b ripple counters.

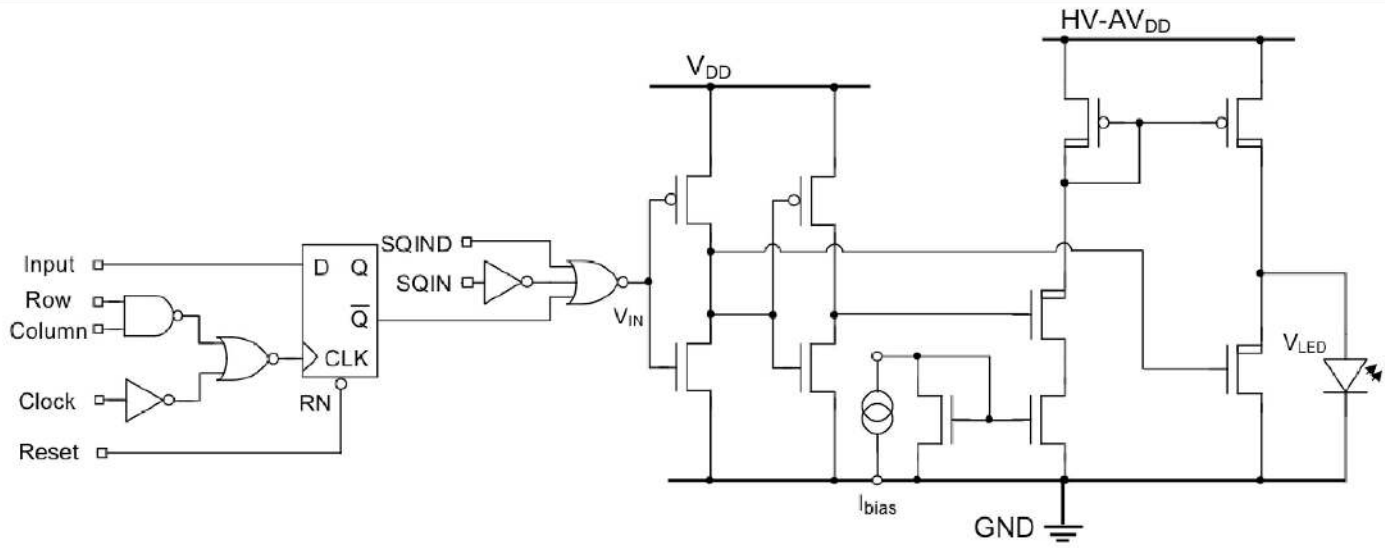

Figure 8.3.5: Micro-LED driver circuit with addressing and pulse generation logic.

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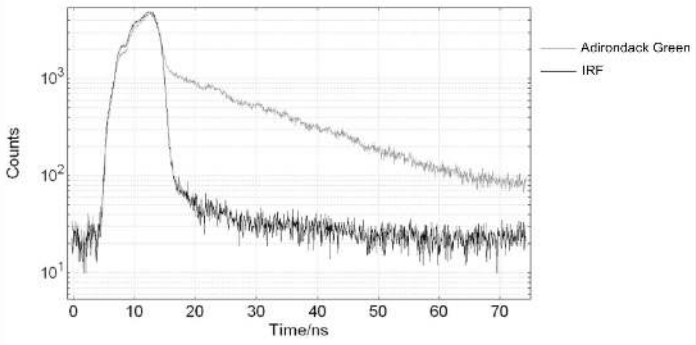

Figure 8.3.6: Fluorescence decay of Adirondack Green quantum dot sample captured using the direct observation SPADs under the micro-LED array and processed off chip using TCSPC hardware.

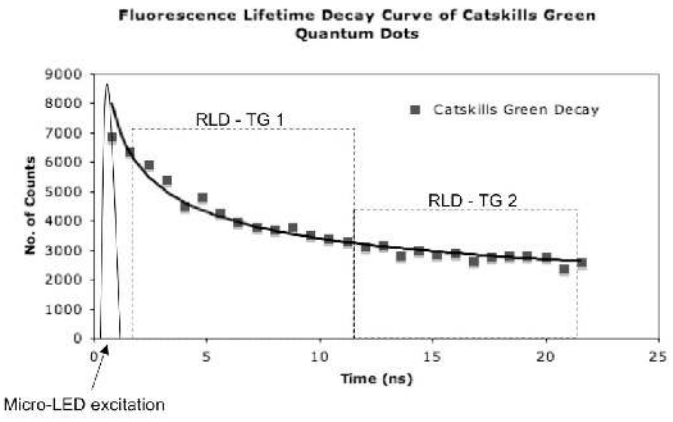

Figure 8.3.7: Fluorescence decay of Catskills Green quantum dot sample captured using the on-chip circuitry, in 26 time gates, each 800ps wide. An illustration of how the same decay is measured using the 2-gate RLD is also provided.

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