

Photon Counting Sensors for Medical and Biophotonic Applications

ULTRA SENSITIVE DETECTORS WITH SILICON PHOTON COUNTING SENSORS

Biophotonic analysis methods are often based upon the measurement of small light quantities. A new generation of photon counters is not only cheap and easy to integrate but propagate in addition the development and miniaturisation of powerful measuring devices.

**STEPHEN BELLIS
CARL JACKSON
ANDREAS KÖNIG**

VLow light sensing is an established field that makes many of the biomedical discoveries of today possible. The combination of fluorescent probes, high speed lasers and photon counting detectors has resulted in many new techniques that have been successfully used to develop a wide range of ultra-sensitive analytical tools. These have been successfully deployed in genomics, proteomics, cell imaging, mass spectroscopy and high throughput screening to name just a few. More recently, these techniques have received wide attention through the human genome project and the development of DNA testing culminating in billboard level product advertising in some markets.

Low light sensing today is predominantly based on the photo-multiplier tube (PMT) for single point detection and on the charge coupled device (CCD) for array based detection. The analytical equipment is typically non-portable and normally located in well equipped central labs. Whilst there has been research to show the viability of point-of-care and point-of-use instrumentation this has been slow to develop and migrate into mainstream use. This can be blamed on many factors, but a key issue has been the lack of commercially available, inexpensive, miniature and high sensitivity optical detectors combined with innovative detector systems. Recent developments in silicon photon counting detectors will revolutionise the manner in which medical diagnostic testing is performed. They will generate a wealth of new point-

of-care and point-of-use instrumentation customised for the individual within the required detection environment. These developments centre on the use of novel low light sensors that can be implemented either as single high-speed detectors or as large area PMT replacements or within a photon counting array in emerging in-vivo detector systems.

voltages, complicated drive electronics) they have only penetrated a small portion of the total market for biological photon counting. Intensified CCD (ICCD) and the electron-multiplying CCD (EMCCD) have emerged in the past decade, however, these devices are expensive and require extensive cooling. Also with new emphasis by manufacturers towards high volume, lower cost diagnostic equipment, these detectors are



SensL photon counting platforms all incorporate time binning and standard USB interfaces to computer based graphical user interfaces. Left you see an single photon counting sensor, below a four channel counter which is expandable for up to 16 channels, including cross and auto correlation for each channel.

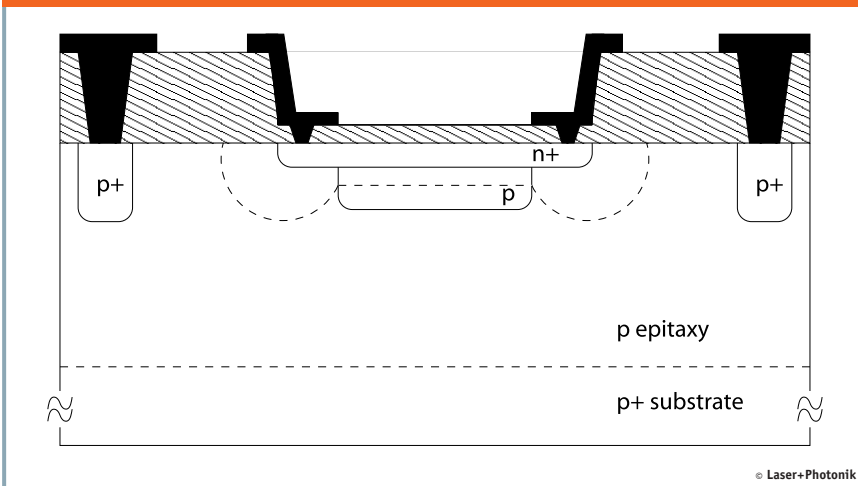
Photon Counting

Traditional photon counting requires vacuum tube based PMTs or fast micro-channel plate (MCP) based detectors. Despite their low quantum efficiency, fragility and high operating voltages, a large variety of testing protocols have been developed that specify these detectors. First generation silicon based photon counting detectors have generated interest but due to serious limitations (such as non-standard manufacturing, high

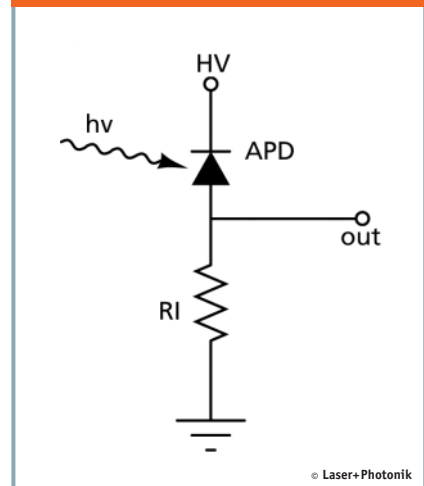


largely inadequate. Previous detectors (that have been very suited to the development of commercial scale laboratory equipment) do not provide the combination of low po-

Photon Counter Set-up

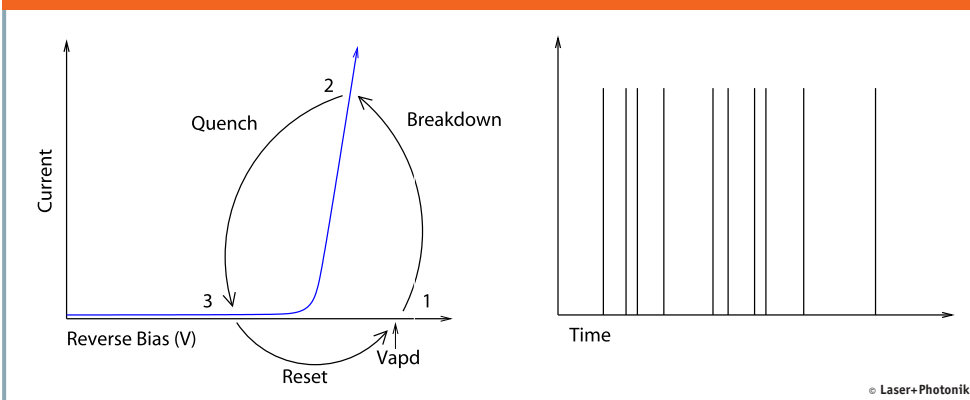


Connection Scheme



1 The APDs have a shallow junction for blue sensitivity, low operating voltage, and are immune to damages from ambient light. Since they are fabricated in a silicon CMOS process, fully integrated system on a chip is possible. In Geiger mode a reverse bias HV is applied at or near below the breakdown voltage of the device. Breakdown causes a sharp current surge but this is quickly quenched in microseconds using passive circuitry or in nanoseconds using active circuitry. The result is a series of current spikes that can be statistically analysed to give a photon count rate over time.

Current Peak



photon must strike the detector and generate an electron/hole pair. This carrier must drift to the high field multiplication region and undergo impact ionisation and avalanche breakdown. The avalanche breakdown process causes a large current to flow in the detector. Once the photon has been detected, the voltage across the diode must be reduced and the charge in the diode allowed to dissipate before the voltage can be re-

stored and photon counting operation can be resumed. This cycle of breakdown, quench and reset is shown in **figure 1**. In typical photodiodes the current increases rapidly for any increase in voltage beyond the breakdown voltage. However, it is possible to bias above the breakdown voltage for a finite period of time before avalanche breakdown occurs. When the breakdown occurs it is necessary to use external means turn it off. A simple method of turning off the breakdown is the passive quench circuit shown in **figure 1**. This can be easily fabricated on-chip using the poly-silicon layers inherent in standard integrated circuit manufacturing processes and leads to a high performance low-cost optical detection module or array.

The Geiger-mode APD

The new second generation detectors are silicon photon counting diodes operating in Geiger-mode. Traditionally silicon detectors that are used for optical detection are PIN, PN or APD photodiodes. These devices are reverse biased to allow the collection of photo-carriers induced by photon absorption in the substrate which are then collected and appear as a current at the output of the detector. The photon counting diode has a similar device structure to these less sensitive photodiodes, but has been specially pro-

cessed to provide a high internal gain mechanism to overcome the noise inherent in the traditional photodiode output circuitry. This is significant because the limiting factor using a typical photodiode is the Johnson noise at the output. Furthermore, having a low noise, high gain amplifier at the point of detection eases the constraints on the output electronics. In a Geiger-mode APD the gain is greater than one million providing a large current pulse at the output of the detector with each incident photon. A schematic representation of the detector used to accomplish this is shown in **figure 1**.

This diode structure differs from first generation technology in that it is manufactured solely with integrated circuit compatible process techniques. Thus the detector can be fabricated with integrated circuits providing the ability to have high sensitivity with on-chip added functionality where required. To register a count, a

photon must strike the detector and generate an electron/hole pair. This carrier must drift to the high field multiplication region and undergo impact ionisation and avalanche breakdown. The avalanche breakdown process causes a large current to flow in the detector. Once the photon has been detected, the voltage across the diode must be reduced and the charge in the diode allowed to dissipate before the voltage can be re-

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The quenching cycle-time can be dramatically improved by using the more complicated "active" quenching technique which can facilitate count rates from one to ten million counts per second. ▶

▶ As an indication, this level of sensitivity allows signal resolution from $7E-7$ to 7 pW of 650 nm light. The digital nature

INFO: Photon Counter

The new generation of silicon based photon counters of SensL provide features which influence the performance of the detectors:

■ **Lower operating voltages.** The detectors can be operated at voltages as low as 30V whereas a PMT typically operates at over 1000V.

■ **IC technology.** The detectors are manufactured using a CMOS compatible process and can therefore be integrated with the drive, counting and communications circuitry helping achieve the miniaturisation required and eliminating high cost packaging.

■ **Small size.** The sensor must be small enough for implantation in vivo but also highly robust. These silicon sensors are much smaller and lighter than PMTs and are immune to damage from spurious light sources.

■ **High Speed.** Picosecond level response times allow them to register photons with the high resolution required for modern molecular probes.

■ **High Quantum Efficiency.** High quantum efficiency across the visible spectrum allows the use of low cost optical excitation sources that can decrease system size and cost.

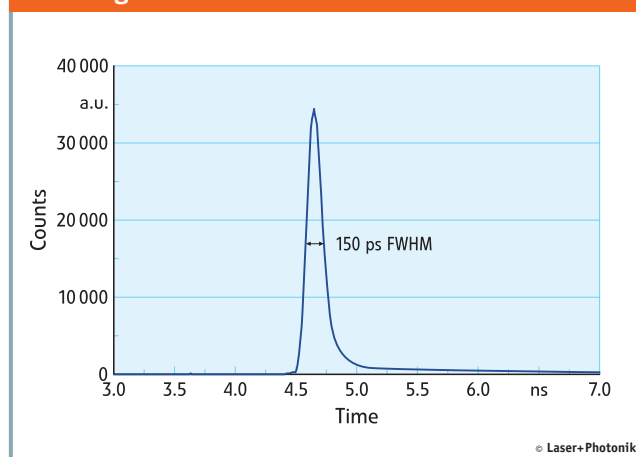
■ **User friendliness.** PMTs need to be shaded from ambient light and magnetic fields when the high voltage is applied to them (terrestrial fields cause measurable errors). In SensL's next generation detectors, no such precautions need to be taken to protect and operate the devices.

■ **Manufacturing cost.** SensL solid-state sensors are manufactured with the same fabrication processes as solid-state integrated circuits and hence have a lower high volume cost base and can be mass produced in batch processes.

of the photon counting detector output is shown in **figure 1**. Each photon into the detector produces an output that can be directly correlated to the photon arrival time. The SensL second generation devices are designed to maximise the photon timing resolution making it possible to accurately determine photon arrival time (imperative for the fast fluorescence signals typically used in advanced molecular probes). This timing resolution is also impacted by the jitter of the output pulse

APD detector capabilities of high sensitivity, low power, robust operation and the ability to batch fabricate as an integrated circuit, new electronics and module configurations are required. As these devices operate with high timing precision providing digital outputs requiring effective bandwidths up to 10 Mhz, their system integration requires careful planning of electronics and the capability to take advantage of digital signal processing techniques. To help end-users SensL has deve-

Jitter Signal



2 Detector module timing jitter measured from a standard $20 \mu\text{m}$ based photon counting detector system. A fast timing card and a pulsed laser system is used to measure the timing accuracy of a photon counting system. Reduced timing jitter is important for fluorescence lifetime measurement

which is therefore of equal importance in determining the photon arrival time. A typical timing jitter is shown in **figure 2**. Typically 50 to 150 ps can easily be obtained which compares to around 600 ps for a PMT and approx 350 ps for first generation silicon detectors.

The Geiger-mode APD structure is fabricated with the doping of the junction allowing for low voltage operation of the order of 30 to 40 V. This eases the constraints on the power supplies required for the development of point of care devices. The detector's shallow junction, which is inherent in integrated circuit processing, gives the detector optimal sensitivity to wavelengths in the range from 400 to 850 nm. The responsivity for a typical device optimised for red detection is shown in **figure 3**. This curve can be tuned for the desired wavelength of interest and the use of batch fabrication and thin film precision antireflection and filter coatings allow the creation of devices with optimised sensitivity.

Novel Electronics

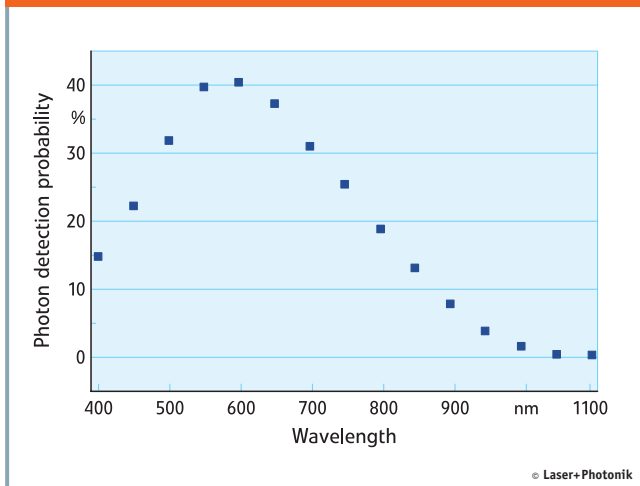
To take full advantage of the Geiger mode

developed and commercialised a novel product platform that allows the full features of the Geiger-mode APD to be exploited (**figure 4**). In its photon counting product line, SensL has incorporated an active quench circuit, Peltier thermo electric cooler controller (both for cooling and detector noise reduction), detector power supply and full interface electronics. The design is built around a micro-controller to monitor and control all the functions of the module and detector. A USB PC interface is provided to facilitate a graphical user interface control. This allows setting

CONTACT

SensL HQ,
Blackrock, Cork / Ireland,
Tel. +3 53 /21 /4 35 04 42,
Fax +3 53 /21 /4 35 04 47,
www.sensl.com

Laser 2000 GmbH,
82234 Wessling,
Tel. 0 81 53 /4 05 -0,
Fax 0 81 53 /4 05 -33,
www.laser2000.de

Detection rate at several wavelengths

3 Detection rate in dependance of the wavelength. The detector is optimised for visible wavelength light detection. The highest value is measured at 600 nm

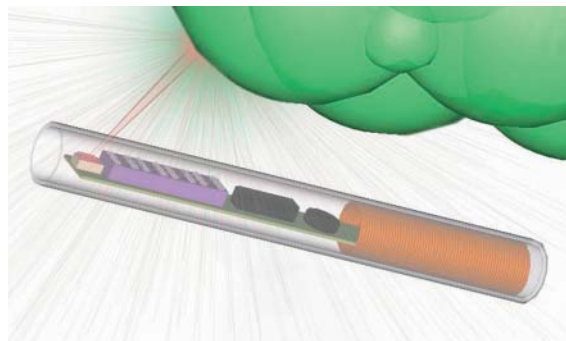
of operating parameters such as detector voltage and internal timing parameters to optimise the detector for a specific application.

The SensL photon counting product line is built around a coordinated detector platform. All standard platforms share either a 20um or 50um detector mounted on a two-stage Peltier thermo electric cooler. Versions are available with a different number of detectors ranging from single detector units to 16 channel detector units for the most demanding applications. Specially selected detectors are available which operate with low dark count (100 counts per second) at room temperature and require no cooling. Larger device areas and custom shapes (such as rectangular which is ideally suited for microfluidic slit applications) are available. Unique to all of the detector platforms is integral time binning with updated count rates at a number of different bin times. This provides the ability to have 1ms count rate bins updated directly to the graphical user interface and precision photon timing that allows time bins of 2.5ns. This allows multichannel scaling or time tagging providing direct autocorrelation and cross correlation between detector channels. Signals are stored and processed in real time with the received information displayed directly on a computer graphical user interface or integrated into the users software. For most applications this negates the need for any external counting electronics or interface cards.

For original equipment applications a miniature photon counting platform provides a unique feature in that quench,

peltier and computer interface electronics are built on multiple boards that can be matched together as required. Only a top level quenching board is required for operation and control of the Peltier cooler can be managed by the system designer and built directly into the system. A standard bus system means that they are automatically enabled and operational as soon as they are connected.

These systems are all built around fully



4 SensL is working on next generation in vivo medical devices. The implantable fluorescence detector concept aims to miniaturise existing photon counting circuitry and integrate with the latest VCSEL technology and the DVS wireless reader system from SensL

modular design concepts and can be easily adapted through firmware and software changes to integrate easily into point of use and point of care applications. For future generations of point-of-use applications one exciting concept that the new generation of photon counting detectors allows is the ability to migrate photon counting technology into high volume, high precision detection applications. Often the use of photon counting detectors and procedures can eliminate costly optics and high power sources from medical equipment and provide higher levels of sensitivity at the same time.

Implantable Sensors

Using an existing radio telemetry platform manufactured by Sixel1 a photon counter and a VCSEL laser have been integrated into an implantable platform 2mm in diameter and 18mm long. The device is currently implanted into or close to solid-mass tumors using minimally invasive procedures. This is made possible by the low voltage, robust photon detection platform that the SensL detector allows. An example of this system is shown in Figure 5. For a successful implantable fluorescence sensor, it is key to have a robust telemetry platform as the backbone of the system. Such a platform exists in the Sixel1 DVS that is currently under development for the detection of radiation dosage during cancer irradiation programs. The key to the system is to merge the photon counting technology platform from SensL with the implantable telemetry solution. The sensor must catch as many of the fluorescence photons as possible while differentiating the shorter wavelength VCSEL emission and any stray excitation photons that may impact the active area of the APD. During APD fabrication, micro-lensed filters are deposited onto the wafer to block the VCSEL excitation band. The mi-

cro-lensing serves two purposes - to collimate the incoming light for correct filter operation and to expand the capture window of the APD device. Dyes, such as Alexa Fluor 647 (Invitrogen), act as a marker to show, for example drug uptake and a 650 nm wavelength VCSEL provides for a safe light source suitable for excitation of this dye. The VCSEL chip is small (300 microns x 300 microns x 100 microns), requires only one tenth of the operating current of edge emitting lasers, can be fast pulsed (< 1ns), essential for low power operation, and operates efficiently at body temperature.

This in vivo approach will complement current approaches to molecular ima- ▶

ging (eg. PET imaging). Unlike imaging methods, however, an implanted sensor system can be used in an office setting rather than a hospital radiology suite, thus allowing medical oncologists to be better integrated with their patients. ■

Summary: First achievement

The second generation of silicon based photon counting detectors are opening up new applications in the medical point of care and point of use market. New detector designs combined with user-friendly software interfaces and high precision photon timing is providing users of existing low light level detection equipment easy paths to adoption of photon counting techniques. SensL has provided a fully scalable path for use in today's large scale medical testing equipment as well as the next generation of fully integrated point of use systems. The ability to scale to fully implantable in vivo detection systems is currently underway and opening the doors for the development of new and exciting systems that use this unique detector technology.

AUTHORS

STEPHEN BELLIS is Senior Design Engineer and

CARL JACKSON is CTO with SensL Technologies in Blackrock, Ireland.

ANDREAS KÖNIG is responsible for Sales & Marketing with Laser 2000 in Wessling/Germany.