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Hybrid photodetector for single-molecule spectroscopy and microscopy

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Abstract

We report benchmark tests of a new single-photon counting detector based on a GaAsP photocathode and an electron-bombarded avalanche photodiode developed by Hamamatsu Photonics. We compare its performance with those of standard Geiger-mode avalanche photodiodes. We show its advantages for FCS due to the absence of after-pulsing and for fluorescence lifetime measurements due to its excellent time resolution. Its large sensitive area also greatly simplifies setup alignment. Its spectral sensitivity being similar to that of recently introduced CMOS SPADs, this new detector could become a valuable tool for single-molecule fluorescence measurements, as well as for many other applications.

Keywords

hybrid photodetector; single-molecule; fluorescence; spectroscopy; microscopy; lifetime; afterpulsing; TCSPC; photocathode; FCS

1. INTRODUCTION

1.1 Single-molecule fluorescence spectroscopy and microscopy

Single-molecule fluorescence spectroscopy and microscopy (SMFSM) refers to a now widespread set of techniques having in common to deal with the usually very weak fluorescence signal emitted by individual fluorophores. Several reviews have been published over the past few years describing the various fields of applications of this general approach and the typical setups employed in these experiments^{1–3}. We will therefore limit ourselves to a very basic reminder of the main constraints encountered in SMFSM. The interested reader will find more detailed background information as well as an extensive list of references in the review referred to above. In particular, we will emphasize the constraints imposed by current

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detector technologies, a topic we have recently addressed in more details⁴. This will allow us to justify our work on the new HPD detector introduced by Hamamatsu Photonics, which turns out to be an attractive detector for SMFSM in a number of aspects.

Single organic fluorophores emit a single fluorescence photon at a time upon excitation, a process which is classically characterized by an absorption cross-section, a quantum efficiency and a fluorescence lifetime. Due to possible transition to the triplet state, which results in no emission of visible photons, the number of emitted photons per unit time is not directly proportional to the excitation intensity, but instead saturates to a maximum value. Therefore, the rate of photon emission of a single fluorophore is limited. Compounded to this constraint is the existence of photobleaching processes resulting in loss of fluorescence, and whose probability is usually increased at high excitation intensity. Neglecting saturation and photobleaching, another important aspect is that excitation intensities have to be significantly larger than the resulting emitted intensities (due to small cross-sections). Not only do these excitation photons have to be spectrally rejected to not bury the signal into noise, but they can excite the fluorescence of nearby impurities or result in nonnegligible Raman scattering signal from the solvent, leading to a background signal that increases with excitation intensity. The bottom line of these considerations is that SMFSM relies on an efficient reduction of all background sources and an efficient collection of the emitted fluorescence photons to obtain good signal to background and signal to noise ratios (SBR and SNR). The last point involves detector sensitivity, but also efficient optical coupling of the detector to the emitter. Finally, the detector readout process should introduce a minimum of additional noise.

1.2 Detectors for SMFSM

Two different types of detectors are used in SMFSM: point detectors and wide-field detectors. Until recently, point detectors such as photomultiplier tubes (PMT) or single-photon counting avalanche photodiodes (SPAD) were the only one to provide the ultimate performance in terms of readout noise, by being able to function as single-photon counting devices. In addition to this beneficial characteristic, they also provide the possibility to not only count the number of photons, but precisely measure their arrival time (macrotime). This allows studying intensity fluctuations at very short time scales, and, in the case of pulsed excitation, to get access to the time elapsed since excitation of the fluorophore (or nanotime). This latter information is of particular interest when studying the environment of a fluorophore, as the presence of additional non-radiative relaxation channels reduces the measured fluorescence lifetime. Typical lifetimes being in the ns range, precise measurement of this information requires detectors having impulse response function (IRF) in the sub-ns range. Point detectors are employed in setups which collect signal from minute volumes, typically the diffraction-limited excitation spot of a high-numerical aperture microscope objective lens (volume $\sim$ fl). The main advantage of this geometry is in reducing the background contribution from out-of-focus molecules. Used with very low concentrations of molecules in solution (<100 pM), it allows detecting single diffusing molecules as well-separated bursts of 100's of photons as they rapidly (~ 1 ms) pass through the excitation volume. Used with more concentrated solutions, in which bursts corresponding to individual molecules start to overlap or become indistinguishable, this

configuration allows the rapid measurement of intensity correlation curves (autocorrelation of a single detector or cross-correlation between several detectors). The variants of this fluorescence correlation spectroscopy (FCS) approach are reviewed in ^{5, 6}. A third approach using point detector is confocal microscopy (of single molecules). Here, the sample is raster-scanned through the diffraction-limited volume using a piezo-scanner to form an image pixel by pixel. The main difference with standard confocal microscopy is the dwell-time (or time per pixel) required in single-molecule confocal imaging. Generally, several ms are necessary to accumulate enough photons to obtain an image with good SNR. For this reason, single-molecule confocal microscopy is usually limited to surface-attached molecules, or static samples. An advantage of this approach over diffusion-based experiments is that an individual molecule can be observed over longer durations (usually until it photobleaches, which occurs after several seconds or minutes). However, only one molecule can be studied at a time, severely reducing the throughput of this technique.

Wide-field detectors will not be discussed in any details in this article, but a typical example is the charge coupled device (CCD) camera and its variants (intensified CCD or ICCD, electron multiplying CCD or EMCCD). Their main advantage is that they allow imaging of many single molecules in parallel, using wide-field excitation. This permits studying triggered dynamic (and possibly irreversible) processes on several molecules at a time, thus providing enough statistics for a meaningful analysis. However, as their name indicates, they rely on accumulating photo-generated charge carriers (electrons) in each pixel, therefore losing all timing information of individual photons. The temporal resolution of the measurement is therefore limited by the readout rate (typically > 1 ms). Additionally, the readout of the accumulated pixel charge adds noise to the signal, reducing the SNR compared to photon-counting detectors. The seemingly irreconcilable characteristics of point-detectors (photon-counting and timing) and wide-field detectors (charge accumulation but parallel measurement on several molecules) will hopefully be combined in future detectors^{4, 5, 7-10}.

1.3 Organization of this article

Section 2 will briefly describe the principle of operation and important characteristics of the new single-pixel hybrid photodetector (HPD) developed by Hamamatsu Photonics K. K. Section 3 will present a comparison of data obtained with this detector and a standard SPAD in 2 typical applications of point detectors for SMFSM: imaging and single molecule diffusion. We will also present some typical FCS measurements obtained with this detector. Section 4 will summarize our results and the possible advantages of this detector for SMFSM.

2. OPERATION AND CHARACTERISTICS OF THE SINGLE-PIXEL HPD

2.1 Principle of operation of the HPD

In developments since the early 1990's, hybrid photodetectors are comprised of a photocathode mounted on top a vacuum tube followed by an avalanche photodiode (APD) operated in its linear gain regime^{11, 12}. A large electric field is created in the tube in order to accelerate the photo-electron generated by the photocathode. The accelerated electron

impacts the avalanche photodiode silicon target, creating thousands of secondary electrons, which are then further amplified by the APD. The overall gain of these devices (number of electrons per photo-electron) can reach 10^5 , resulting in a measurable single-photon signal. These devices have a sensitivity directly related to the material used for the photocathode. Recently, Hamamatsu has extended this technology to GaAsP photocathode, which provides an excellent sensitivity in the visible spectrum (close to 50 % from 500 to 700 nm)¹³. Here we present results obtained with a prototype of this detector (model R7110U-40UF, Hamamatsu).

Compared with the SPAD devices currently used for SMFSM, HPD detectors are less user-friendly, as the signal generated after the two-stage amplification is generally weaker than that of current PMTs for which most time-correlated single-photon counting (TCSPC) electronics have been developed. Having a weaker signal means that additional amplification is needed in order for these electronic modules to process the signal satisfactorily. Here we used a high speed amplifier providing an extra gain of $\times 63$ (Model C5594-12, Hamamatsu) directly attached to the output of the detector. For use with non TCSPC devices, such as counting boards or correlators, pulse shaping is required. We tested two different devices for this purpose (1-GHz amplifier and time discriminator, Model 9327, ORTEC and Photon counting unit, Model C9744, Hamamatsu). Both provide a TTL output, the ORTEC module providing in addition a fast NIM output. As we will see later, the fast preamp/discriminator stage is the main limitation we have encountered in trying to test the limits of this detector.

Fig. 1. shows a prototype single-pixel HPD with a fast pre-amplifier (model C5594-12, Hamamatsu) connected to it. The high-voltage supply cables are a reminder that cautious manipulation (and installation) of this detector is warranted. In particular, the body of the HPD should be at a minimum distance of 7 mm from any conductive surface, in order to prevent sparks. The commercial version of the HPD will be enclosed in a protective case, relieving the user from this concern and facilitating mounting on an optical setup. The operating voltages used in these experiments were -8.5 kV for the photocathode, and 385 V for the APD. In this regime, the gain from the tube stage is close to $2,000$, and that of the APD is ~ 60 , resulting in an overall gain of $\sim 120,000$. The power supply for both stages is provided by a single small unit (Model C8238, Hamamatsu) connected to the lab's AC power supply.

The sensitive area of the detector is 3 mm in diameter, greatly simplifying alignment and relaxing somewhat the constraint of having a focused light spot impinging on the detector. However, our experience with a tightly focused light spot (diameter < 70 μm), shows that the sensitivity of the detector depends slightly (~ 7 % peak-to-peak variations) on the exact location of photon impact in agreement with the results of ref.¹³, which were obtained with a larger illumination spot (0.5 mm) and reported less than 10% variation across the detector area.

2.2 Characteristics of the HPD

2.2.1 Quantum efficiency—The quantum efficiency of the unit we tested is shown in Fig. 2, and compared with other point detectors used in SMFSM. The detector's

characteristics compare well with the CMOS SPADs (PDM and Id 100), although they are inferior to the Perkin-Elmer device above 500 nm. Note in particular that the GaAsP has no sensitivity above 750 nm, contrary to the Si-based detectors. We performed crude comparisons of the detection efficiency of the HPD and the SPAD using a sample having a broad emission spectrum, filtering the signal with different band pass filters and splitting the resulting photons with a non-polarizing beam-splitter redirecting the light to the HPD and SPAD respectively. Taking into account the emission spectrum of the sample, the transmission characteristics of the filters, we found a reasonably good agreement between the measured and expected sensitivity ratios between the two detectors in the different spectral windows (data not shown).

2.2.2 Pulse output—The single-photon output of the detector after amplification by the C5594-12 unit, detected by a fast digital oscilloscope (TDS 3052B, Tektronix) is shown in Fig. 3. The pulse is very clean and narrow (full width of ~1 ns), but varies in amplitude, as expected. This amplitude variation, which is of little importance for regimes where a single photon impacts the photocathode at a time, is small enough to actually distinguish single-photon from n-photon events ($n < 7$) as has been shown previously¹³. More importantly here, it shows that the detector has a deadtime of a few ns at most. This is in stark contrast with SPADs, in which it is necessary to quench the avalanche generated for each detected photon, since the APD is operated in the non-linear regime in SPADs, a process which usually requires 30–40 ns to complete. This minimal deadtime means that saturation effects appear at higher count rates than for SPADs, a potential advantage for HPDs in single-photon counting at very high count rates. Note that, similarly to PMTs or some recent CMOS SPADs, HPDs can be used in DC mode, by giving up single pulse detection, and simply recording the output current. This has potential application for non single-molecule (photon counting) confocal microscopy.

The counting units used in this work are characterized by minimum pulse pair resolution of 10 ns (9327, ORTEC) and 25 ns (C9744, Hamamatsu) respectively. Unfortunately, the better pulse pair resolution of the ORTEC module is only available using the fast NIM output, as the TTL output has a fixed duration of 100 ns. Since none of the TCSPC modules we had access to (and would have accepted a fast NIM input) had a deadtime shorter than 100 ns, we could not take advantage of this better performance to test the pulse resolution of the detector itself. Instead, we used the C9744 module of Hamamatsu and its TTL output, with a pulse width of 10 ns, and studied the HPD response using either a PCI-6602 counting board (National Instruments) with a timing resolution of 12.5 ns (and a corresponding pulse pair resolution of 12.5 ns), or a Flex02 correlator (Correlator.com) with a timing resolution of 1.56 ns and identical pulse pair resolution. Note however that in both devices, a minimum pulse width of ~5 ns is necessary to ensure detection, plus a similar minimum pulse separation. We will get back to these specifications when discussing our FCS results.

2.2.3. Dark count—The HPD is operated at room temperature without cooling. Being based on a photocathode, spontaneous dark counts are expected and actually measured. The average dark count measured in our lab was around 1.5 kHz, but this value could reach 3–4 kHz if the module had been exposed to bright light (high voltage power supply turned off!)

just prior to making this measurement. In practice, it would take a few minutes in the dark for the HPD to recover its nominal dark count rate of ~ 1.5 kHz. Although high compared to typical SPAD dark count rates (~ 50 – 500 Hz depending on the model or unit), one has to remember that these devices are Peltier cooled. Potential improvements of the HPD dark count rate may result by incorporation of such a feature. However, in practice, it is rare that the single-molecule sample's background is not itself of the order of a few kHz, so that this relatively high dark count rate is in practice not a major issue.

2.2.4. Afterpulsing—The HPD is based on an APD operating in its linear regime, which let us to expect that it does not suffer from one of the drawbacks of SPADs, namely the presence of spurious signals originating from trapped carriers thermally released a few 100 's of ns to μ s after a real photon conversion, or afterpulses¹⁴. In SPADs, only a small percentage of real pulses are followed by afterpulses (the afterpulsing probability), but over large period of times and for high count rates, this can amount to a larger number of counts that can skew sensitive measurements.

A simple way to study afterpulsing probability is to plot a histogram of time interval between successive pulses. For a detector with no afterpulsing detecting the signal from a sample emitting at a constant average rate, this histogram should be decaying exponentially, as expected for a shot noise limited process (Poisson process). In the presence of afterpulsing, a single exponential is not sufficient anymore, and an additional component, giving the typical interval between a pulse and its after pulse will appear. This phenomenon is readily visible in Fig. 3A, which shows such an histogram as well as a multi-exponential fit for a sample emitting at an average rate of 40 kHz $\sim (22 \mu\text{s})^{-1}$ detected by the SPAD (the emitted intensity actually decays linearly from 55 kHz to 35.5 kHz over the 5 min duration of the recording). In addition to a component with a time scale of $\sim 25 \mu\text{s}$ corresponding to the average count rate, a non-negligible component with a time constant of 133 ns is clearly visible. In contrast, the HPD histogram measured simultaneously can be fitted perfectly with a single exponential with time a constant corresponding to the inverse count rate, demonstrating the absence of afterpulsing in this detector. We will present further evidence of this in section 3.3.

2.3 Fluorescence lifetime measurements

The timing resolution of the HPD has been studied in detail in ref. ¹³ and shown to be on the order of 60 – 90 ps, depending on the spatial spread of the photon on the detector. In our setup, described in ref. ¹⁰, we are unfortunately unable to reach this level of timing resolution in actual fluorescence measurement due to a jittery PIN-photodiode used to pick up the excitation laser pulse. Nonetheless, Fig. 5 shows a typical fluorescence decay of FITC excited by a 4.75 Mhz sub-ps laser at 442 nm. Due to the filter set used to detect FITC (530DF30) the Raman signal of water (526 nm) is detected as an IRF-limited peak. The full curve is perfectly fitted by a single exponential component convolved with a Gaussian of $\sigma = 89.1$ ps plus a Dirac component (convolved with a Gaussian) for the Raman signal. The IRF has therefore a FWHM of 210 ps, which is in comparison smaller than the corresponding IRF previously measured with another detector with a timing resolution of 80

ps¹⁰. We conclude from this measurement that the timing resolution of the HPD is better than 80 ps, in agreement with the measurements of ref. ¹³.

3. SINGLE-MOLECULE EXPERIMENTS USING THE HPD DETECTOR

Having verified that the HPD has similar characteristics to those of more common detectors in SMFSM, we tested its performance in some typical experiments.

3.1 Diffraction-limited imaging using stage-scanning confocal

As mentioned in the introduction, one of the many applications of single-photon counting point detectors in SMFSM is stage-scanning confocal microscopy. Stage-scanning using a nm-resolution closed-loop scanner allows images with arbitrary size as well as pixel size to be acquired, the only limitation being the scanning speed of the scanner, which is limited by its mechanical resonance frequency (on the order of a few 100's Hz). This means that dwell time per pixel in the μ s range are not realistically achievable, but since single molecules emit very few photons, minimum dwell times in the ms range are usually required.

For molecules fixed on a surface, with no out of focus background source, as it is the case for dry samples, the use of a pinhole is not necessary and actually not recommended to maximize the collected signal. In addition, the small sensitive area of SPADs (20–200 μ m in diameter) acts as a pinhole. Due to the larger size of the sensitive area of the HPD, we should therefore see a difference in the images obtained by an HPD without pinhole and those obtained by a SPAD without pinhole. Fig. 6A illustrates this difference with images of a 100 nm diameter fluorescent bead excited with the 488 nm line of an Ar ion laser and imaged on a glass coverslip with a NA 1.2 water immersion 63X objective lens (P/N 44 06 68, Zeiss). These images are for all practical purposes the convolution of the excitation PSF with the emission PSF defined by the sensitive area of the detector (HPD: 3 mm diameter, SPCM-AQR 14, Perkin-Elmer, $\sim$ 180 μ m diameter sensitive area). The SPAD image is visibly less extended vertically, possibly indicating clipping of the imaged formed at the SPAD when moving the sample out of focus. Except for this difference, there is no apparent loss of resolution in the images formed by the HPD.

Next we examined the effect of the different detector sensitivities on single-molecule imaging. Fig. 6B shows an image of quantum dots (or qdots) emitting around 565 nm (Qdot 565 Streptavidin conjugate, P/N Q10131MP, Invitrogen) imaged simultaneously by a SPAD and a HPD, using a non-polarizing beam-splitter cube to split the emitted photons evenly between the two detectors. According to Fig. 2, we should expect a $\sim 2 \times$ larger signal in the SPAD image. The measured difference (obtained by fitting the PSFs by a Gaussian as described in ref. ¹⁵) is slightly larger, possibly due to the fact that the emission filter being relatively broad (580DF70), we might have imaged redder emitting qdots, a region of the spectrum where the SPAD has close to twice the QE of the HPD. The main observation, however, is that, as expected, the larger dark count of the HPD (~ 2 kHz in this image) reduces the contrast of weaker signal (upper left image of Fig. 6B). This effect does not prevent the detection of these weak signals, as shown by the images in the lower panel of Fig. 6B, in which the lower threshold has been adjusted to 2 KHz.

3.2 Detection of single diffusing molecule

Another popular use of single-photon point detector in SMFSM is in experiments involving freely diffusing molecules in solution. In this geometry, using a pinhole is recommended to reduce the probability to collect photons emitted by molecules diffusing at a distance from the focus. At low concentration (<100 pM), molecules diffusing through the excitation PSF are detected as brief bursts of fluorescence, whose duration and intensity depend on the exact diffusion path of the molecule as well as on the excitation intensity. Contrary to surface imaging, where low excitation intensities are recommended to allow for long time-scale imaging, high excitation intensities are preferable in the diffusing geometry, in order to collect as many photons as possible during the brief transit of each individual molecule. Fig. 7 shows 20 s fluorescence time traces binned with 1 ms resolution from an Alexa Fluor 488 (Invitrogen) sample in water (left panel) and a Qdot 525 ITK (Invitrogen) sample in PBS 1X. Both samples were excited with the 488 nm line of an Ar ion laser coupled to a NA 1.2 water immersion 63X objective lens, and the signal collected through a 100 μ m pinhole and a 530DF30 filter. The signal was split evenly between the two detectors (HPD: upper traces, SPAD: lower traces) by a non-polarizing beam-splitter cube. Overall the two detectors collect the same number of bursts having similar intensities, as expected from the characteristics of the two detectors in this spectral window (Fig. 2). Interestingly, as argued previously, the higher dark count rate of the HPD is not noticeable, being of the order of magnitude of the sample background.

Although a sample with a redder emission spectrum would be less efficiently detected by the HPD than the SPAD, these data show that the HPD is a good detector for single-molecule detection.

3.3 Fluorescence correlation spectroscopy

One of the main differences between HPD and SPAD is the absence of afterpulsing documented in section 2.2.4 and Fig. 4. Afterpulsing is problematic for experiments which look at temporal correlations between photons detected by the same detector (fluorescence autocorrelation). Although this problem can be somewhat circumvented when using pulsed laser excitation and TCSPC electronics¹⁶, it prevents or at least makes the study of dynamics in the ns- μ s very arduous. In practice, this problem is solved by splitting the signal between two detectors and computing the cross-correlation between these detectors. There are several issues with this approach. First, it severely increases the cost and complexity of the experiment. Second, it requires using two channels of the data recording electronics (correlator or counting board). Finally, it decreases the detected count rate per channel and hence the signal-to-noise ratio.

We checked that the HPD can be used for single-channel FCS by computing the autocorrelation function (AF) of the samples described in the previous sections.

Fig. 8 shows the AF of the two detectors without any incoming signal as computed by a 2-channel correlator (Flex02, Correlator.com) using a multi-tau algorithm. The timing resolution of the correlator is 1.56 ns, but as can be seen, the AF is interrupted below 25 ns

for the HPD (due to the limitation of the C9744 counting unit) and below ~40 ns for the SPAD (due to the SPAD deadtime).

Fig. 9 shows the AF of 20 nM Alexa Fluor 488 sample excited with 90 μ W at 488 nm detected as described in section 3.2. A single AF was computed at full resolution of the correlator and therefore the corresponding AF for the SPAD is not available. The curve is perfectly fitted from 40 ns to 1,000 s with a simple 2D diffusion model with triplet state blinking, yielding reasonable values for the parameters. A similar analysis would of course not be possible with a single SPAD, demonstrating a real advantage of the HPD for this type of applications.

This result shows that a bright signal should provide sufficient statistics to obtain an exploitable AF curve down to the effective deadtime of the HPD (set by the counting unit at 25 ns). Short time lags give access to the antibunching regime of single emitters and we therefore tried to explore the possibility of seeing this antibunching behavior in a single immobilized qdot. In order to maximize the number of collected photons, we used a strategy similar to that described by Hohng & Ha to significantly suppress blinking in qdots¹⁷. Fig. 10 shows an example of time traces obtained in these conditions, as recorded by the SPAD. Some blinking periods are still present. Half of the signal being sent to the HPD, the AF of this signal was recorded by the Flex02 correlator (Fig. 10C). The most noticeable feature of the AF is the presence of oscillations at large time lags, corresponding to the blinking periods. This prevents from fitting individual qdot's AF by a simple model as previously noted by Messin et al.¹⁸. Nevertheless, we used an heuristic model incorporating the exponential antibunching term to fit the first part of the AF (40 ns – 1 ms):

$$g^{(2)}(\tau) = \left(1 + \alpha \left(e^{-(\tau/\tau_1)^\beta} - e^{-\tau/\tau_0}\right)\right) \quad (1)$$

The only potentially meaningful parameter of this fit is τ_0 , which can be identified with the fluorescence lifetime of the qdot at excitation power below saturation. The fitted value of 25.2 ns is in agreement with typical lifetime values for qdots.

4. CONCLUSION

We have tested the new Hamamatsu hybrid photodetector (HPD) based on a GaAsP photocathode, and compared its performance with that of a SPAD (SPCM-AQR, Perkin-Elmer) for single-molecule fluorescence spectroscopy and microscopy. Although its sensitivity in the visible is lower than the Perkin-Elmer SPAD, its quantum efficiency is comparable to that of CMOS SPADs currently available on the market. One potential problem of this detector is its rather large dark count (1–2 kHz), although we have illustrated several applications where it turns out not to be detrimental. This is largely compensated by a complete absence of afterpulsing, which permits the acquisition of autocorrelation function down to the deadtime of the counting electronics. With a much larger sensitive area than SPADs, this detector should simplify (or even suppress the need for) setup alignment. Finally, its excellent timing resolution makes it an ideal detector for the most demanding TCSPC applications.

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Fig. 1.

A single-pixel HPD (right black cylinder shape) with a fast pre-amplifier (C5504-12) connected to it. The red wires are connected to the high-voltage power supply. The metallic plate supports the connector for the APD voltage supply. The sensitive area (not visible under this angle) is located a few mm from the front.

Fig. 2. Quantum efficiency (QE) curves for different detectors used in SMFSM. SPCM-AQR: SPAD manufactured by Perkin-Elmer. PDM: SPAD manufactured by Micron Photon Device. Id 100: SPAD manufactured by Id Quantique. HPD: the detector tested in this study. All curves were obtained from the manufacturers' data sheet.

Fig. 3. Single-photon output characteristics of the HPD after preamplification by the C5594-12. Left: continuous acquisition showing the dispersion in pulse amplitude expected from the gain mechanism. Right: continuous average over 512 pulses. The pulse amplitude is ~ -40 mV, with a width of ~ 1 ns. Notice the moderate oscillations after the main pulse and the low noise level.

Fig. 4. Histogram of time interval between successive photons. A: SPAD, B: HPD. The emitting sample was a 100 nm fluorescent bead recorded for 5 min. During that period of time, the count rate recorded by each detector decayed from 55 kHz to 35.5 kHz (mean: 45 kHz) for the SPAD and from 35.2 kHz to 22.4 kHz (mean: 28.8 kHz) for the HPD. The insets show a detail of the short timelag regime (down to 12.5 ns, the resolution of the PCI-6602 board used in this experiment). The HPD clearly shows no afterpulsing, in contrast with the SPAD.

Fig. 5. Fluorescence decay of FITC excited by a 442 nm fs laser. In addition to the single component decay of 3.94 ns, a peak at time zero corresponding to the Raman signal of water gives a convenient measure of the setup's IRF. The FWHM of the combination setup +HPD is 210 ps. Comparison with previous measurements with the same setup but a different detector implies that the contribution of the HPD is negligible ($< 80 \text{ ps}$). The inset shows a detail of the Raman contribution to the decay, which is perfectly fitted by a Gaussian with $\sigma = 89.1 \text{ ps}$.

Fig. 6.

Stage-scanning confocal imaging. A: 100 nm diameter fluorescent bead imaged without pinhole by a HPD (left) and a SPAD (right). The top image shows a cross section of the bead image along the optical axis (XZ). The bottom image shows the bead cross-section in the plane of focus. Note that the HPD and SPAD images were taken separately. Image dimensions: XY: $4 \times 4 \mu\text{m}$, XZ: $4 \times 10 \mu\text{m}$. B: Single quantum dots (QD565) imaged without a pinhole simultaneously by a HPD (left) and a SPAD (right). The top images show the images using the full dynamic range of the image (0–110 counts/10 ms), emphasizing the higher dark count level of the HPD. The images at the bottom show the same data with a limited dynamic range (20–110), removing most of the dark count of the HPD, but simultaneously masking most of the upper left qdot in the SPAD image, showing that the two detectors have in fact very similar sensitivities. Image characteristics: $4 \times 4 \mu\text{m}$, 50 nm pixels, 10 ms/pixel, 460 nW excitation, 580 DF 70 filter.

Fig. 7. Single-molecule diffusion. Two different samples, Alexa Fluor 488 in water and Qdot525 ITK in PBS 1X, were excited with the 488 nm line of an Ar ion laser and detected through a 530DF30 band pass filter. The 1 ms-binned time traces collected simultaneously by the HPD (upper traces) and the SPAD (lower traces) show similar number of bursts and counts per burst. Note also the similar background count levels, eclipsing the higher dark count rate of the HPD. Alexa 488: 10 pM, 100 μ W excitation. Qdot 525: 10 μ W excitation. NA 1.2 63X objective lens, 100 μ m pinhole.

Fig. 8.

Autocorrelation functions (AF) of the HPD and SPAD dark count signals. Except for noise at very short time lags (<100 ns), the HPD AF is uniformly equal to 1, whereas the SPAD AF shows a non-negligible component below $100 \mu\text{s}$ despite its much lower dark count level. A good fit is obtained with a stretched exponential. Note also the ~ 25 ns timing resolution of the HPD AF compared to the ~ 40 ns resolution of the SPAD AF.

Fig. 9.

20 min AF of a 10 nM FITC sample detected by the HPD through a 100 μm pinhole. The fit is to a 2D diffusion model with blinking and is performed from 40 ns (red leftmost cursor) to 10^3 s (rightmost green cursor). The fitted values are in agreement with the known diffusion time of Alexa Fluor 488 through the diffraction-limited excitation volume, and the triplet state lifetime of Alexa Fluor 488. Excitation power: 90 μW , mean count rate: 14 kHz.

Fig. 10.

A: stage-scanning confocal image of a single qdot. B: 10 min fluorescence time trace of this qdot excited with $6 \mu\text{W}$ at 488 nm recorded with the SPAD. A second qdot is emitting during the first min of recording, as visible from the 3-state nature of the intensity levels. C: Corresponding HPD autocorrelation function acquired simultaneously to the SPAD time trace. The blinking pattern introduces an infinite number of time scales as visible from the oscillations of the AF at large time lags. The red curve represent a fit from 40 ns to 1 ms (between the red and green cursors) by an heuristic model incorporating antibunching at short time scales, with a typical time scale of 25.2 ns.