Technical Note

Performance of the Micro Photon Devices PDM 50CT SPAD detector with PicoQuant TCSPC systems

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These measurements were performed to investigate the ability to use the recently developed thin film CMOS APDs both for ultrafast single photon counting as well as ultra sensitive fluorescence detection in a single molecule spectroscopy system.

Detector

The PDM 50CT module from Micro Photon Devices (MPD) is an actively quenched Single Photon Avalanche Diode (SPAD), specifically designed for photon counting applications.
It is based on CMOS technology and was developed using the long term expertise of Prof. Sergio Cova in Milano together with Microgate Engineering [1,2]. The SPADs of the PDM series are available with three different detector diameters (20, 50 and 100 µm) and can be supplied with a TE cooler to reduce the number of dark counts.
We selected for this test a pre-produced cooled detector module with an active diameter of 50 µm compromising between dark counts and the demands of the single molecule system.

The manufacturer specifications of the PDM 50CT are given to be:

Active area:	50 µm
Timing resolution (FWHM):	<50 ps
Photon Detection Efficiency:	25% @ 400 nm, 47% @ 532 nm, 34% @ 650 nm
Dark counts:	typ. 75 counts per second
Afterpulsing probability:	0.5%
Dead time:	70 ns
Output pulse shape:	TTL and NIM for improved timing resolution

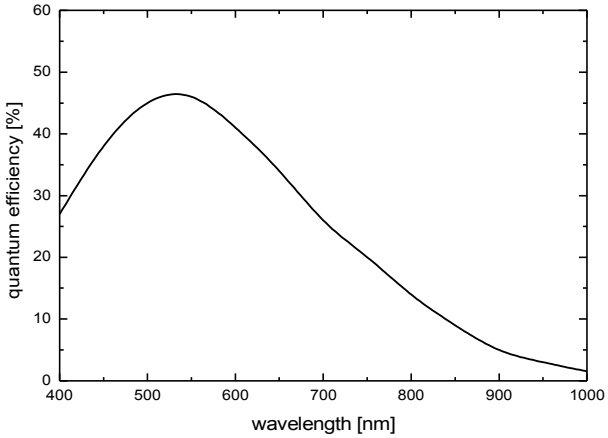

Fig. 1: Quantum efficiency as a function of wavelength as specified by the manufacturer.

Test Setup

The PDM 50CT was tested in two different setups: In order to test the SPAD for potential applications in a single molecule detection system, we integrated the SPAD in the confocal fluorescence microscope MicroTime 200 [3] whereas to investigate the timing behaviour, we used the following setup:

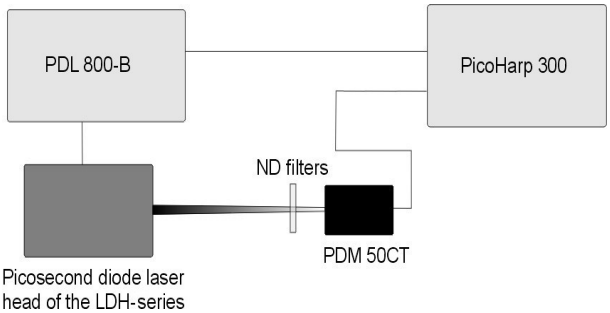

Fig. 2: Experimental setup for the timing analysis of the PDM 50 CT (for details see text).

Light pulses from different laser heads of the LDH-

series, driven by the PDL 800-B generator [4] were directly focussed on the active area of the SPAD. The output power of the laser pulses was regulated by the PDL 800-B and if necessary also attenuated by suited ND-optical filters. The NIM-output signal from the SPAD was used because of its faster timing response and directly connected to the PicoHarp 300 [5], which was used for the time-correlated single photon measurements. The reference signal was directly taken from the PDL 800-B.

To characterize the SPAD different laser heads of the LDH-series with wavelengths between 375 and 670 nm were used. The UV and blue wavelengths are included into the test because they are of high importance in all fields of cell biology and other bioanalytical applications. Furthermore, as the specified timing resolution is close to the value of a typical MCP-PMT, the measurement was repeated under the same conditions but with a R3809U-50 MCP-PMT from Hamamatsu [4] as detector along with the laser head at 670 nm, which shows an exceptionally short pulse width of 32 ps.

Instrument Response Function (IRF)

The Instrument Response Function for the setup in fig. 2 was measured with five different wavelengths between 375 and 670 nm. Some result of this measurement are shown in fig. 3 and all measured IRF widths along with the respective laser pulse widths are summarized in table 1.

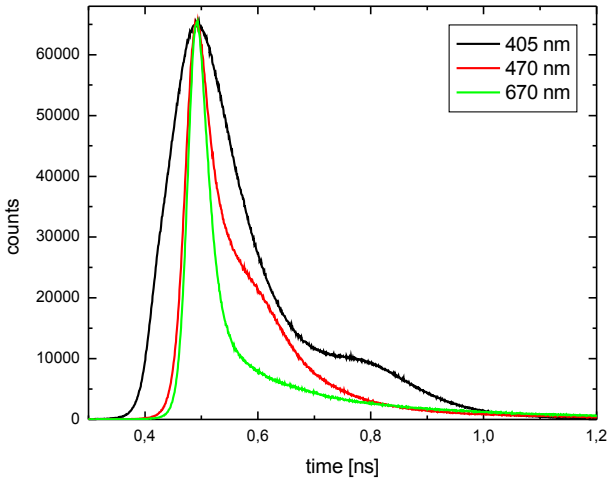

Fig. 3: Wavelength dependence of the IRF.

The results clearly indicate that the IRF width depends on the wavelength. In general, all curves show a fast rise time in the order of 100 ps or below. The trailing edges on the other hand are significantly different and the formation of shoulders can clearly be seen for wavelengths at 470 nm and below. These shoulders are the main reason for the larger IRF width at these wavelengths.

Wavelength [nm]	***IRF, FWHM [ps]***	***Laser pulse, FWHM [ps]***
375	140	45
405	154	55
470	71	56
635	83	66
670	49	32

Table 1: Measured IRF widths at different laser wavelengths.

However, the IRF width is still significantly smaller as compared to photomultiplier tubes (typ. 200 ps), which are typically used in this spectral region and even much smaller as compared to currently used SPADs of the SPCM-AQR series from Perkin-Elmer (typ. 350-500 ps). For wavelengths above 470 nm, the measured IRF width is within the specifications of the PDM 50CT, if one takes the pulse width of the excitation laser into account.

Comparison with MCP-PMT

A comparison of the IRF with similar measurements using a R3809U-50 MCP-PMT from Hamamatsu [6] is shown in fig. 4 and summarized in table 2. As can be seen, the rising edges of both detectors are nearly identical, whereas the trailing edges of the SPAD are longer, which leads to the slightly larger values of the IRF width.

Wavelength [nm]	***IRF, MPD, FWHM [ps]***	***IRF, MCP-PMT, FWHM [ps]***
635	83	73
670	49	48

Table 2: Measured IRF width with the PDM SPAD and a MCP-PMT.

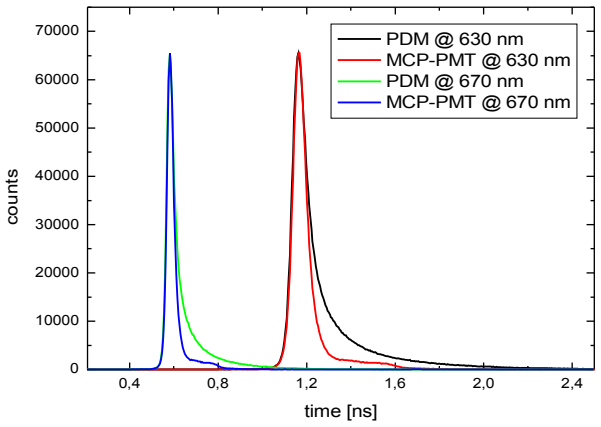

Fig. 4: Comparison between IRFs of a MCP-PMT and the PDM SPAD.

Instrument Response Function as a function of the count rate

All measurements shown above were done with a constant count rate of approx. 100 kHz. Real biological samples in scanning microscopy, however, generate a strongly varying count rate. The currently used SPADs from Perkin-Elmer (SPCM-AQR series) show under these conditions a broadening as well as a temporal shift of the IRF.
In order to test the corresponding behaviour of the PDM 50CT, the measurements were repeated with different count rates using the 670 nm laser for the excitation. The results are shown in fig. 5.

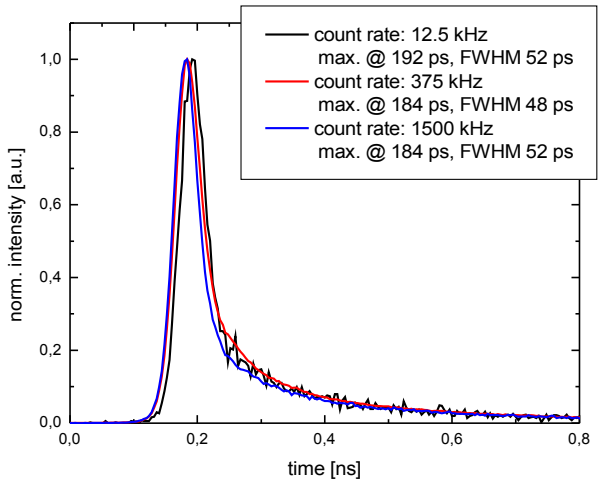

Fig. 5: Influence of the count rate on the IRF position and width.

As can be seen the time shift of the maximum IRF channel is extremely low (less than 10 ps) and also the full width at half maximum (FWHM) of the IRF is almost constant.

Afterpulsing

For correlation measurements a detector with almost no afterpulsing is needed. Unfortunately, the SPCM-AQR SPADs from Perkin Elmer show a significant afterpulsing probability which makes it very difficult to extract correct particle numbers and triplet state dynamics from an Fluorescence Correlation Spectroscopy (FCS) measurement. Therefore, many users currently prefer to do a cross correlation measurement which is free of this artifact but requires a second detection channel and therefore a substantial financial investment.
The afterpulsing measurements were done with a cw-laser running at 635 nm and the TimeHarp 200 [7] in the Time-Tagged Time Resolved (TTTR) mode. The count rate in these experiments was 10 kHz.
The resulting autocorrelation curve in fig. 6 shows only very little afterpulsing. In detail the afterpulsing ceases at about 1 µs, which means that typical diffusion times can smoothly be measured with a single detector.

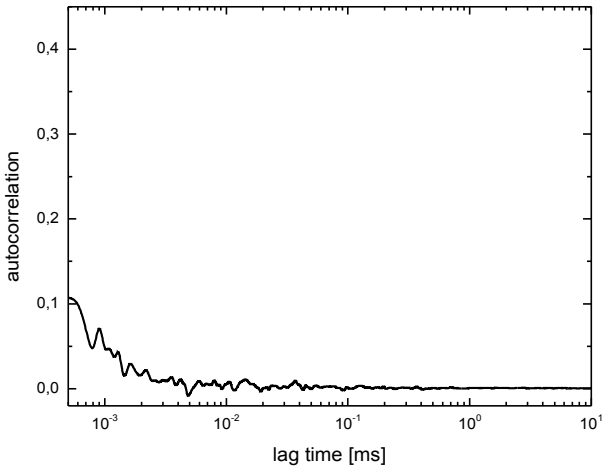

Fig. 6: Autocorrelation function of the PDM 50CT SPAD using uncorrelated light.

Applications in a Single Molecule Detection system

The biggest advantage of a SPAD compared with a MCP-PMT based system is the high quantum efficiency, which is especially important in the field of Single Molecule Detection (SMD). However, SPADs usually have a relatively small active area onto which the emission light needs to be focussed, which puts some demands on the optical setup.
The currently used SPCM-AQR SPADs from Perkin Elmer have an active area of 180 µm, whereas the PDM 50CT has an active area of 50 µm. In a microscope system, like the MicroTime 200 confocal microscope [3], this can be problematic as beampath alignment and confocal pinhole exchange might lead to small beam displacements which in turn can lead to only partial illumination of the SPAD active area.
The currently used SPADs from Perkin Elmer, for example, show a broadening of the IRF for out of center focussing. In order to evaluate the influence of the focus adjustment on the active area of the PDM 50 CT, the light focus was moved diagonally across the active area of the SPAD for different pinhole sizes. The results of these measurements are shown in fig. 7.

It can clearly be seen, that even at a large pinhole size of 200 µm a displacement of approx. 20 µm in total does not change the count rate at all – for the small confocal pinhole size of 50 µm the area of constant count rate even extends to 35 µm. As such relatively large displacements are usually not encountered in a well aligned and stable system, a SPAD with an active area of 50 µm can be very well used in the MicroTime 200 and, in fact, using the sophisticated optics of the MicroTime 200 it was no problem at all to integrate the PDM 50CT into the setup.

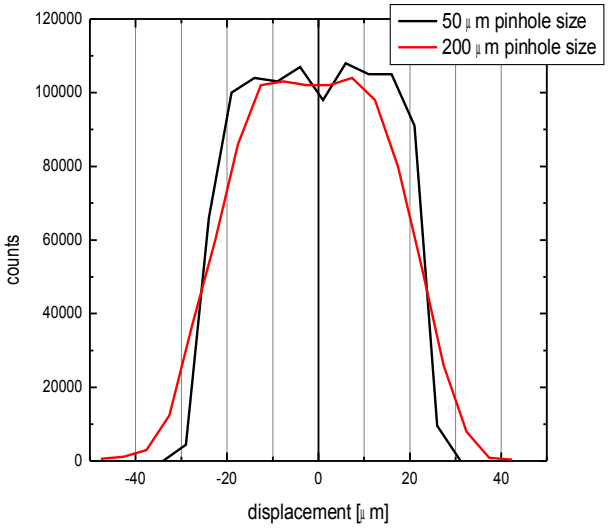

Fig. 7: Signal loss due to partial illumination of the active area. Two different confocal pinholes were imaged and moved across the SPAD active area with a nominal sixfold image reduction between pinhole and active area.

In order to evaluate the possibility of using the SPAD in single molecule applications, Fluorescence Correlation Spectroscopy (FCS) measurements of a very diluted solution ($1x10^{-10}$ M) of freely diffusing Atto655 molecules in water were performed. The excitation wavelength for these measurements was 640 nm and a 100x 1.3 NA objective and a 50 µm pinhole was used. The emission wavelength of Atto655 is in the range of 680 nm. All measurements were done in the TTTR mode of the TimeHarp 200 board, our "workhorse" TCSPC solution for SMD. In principle, the PicoHarp 300 can also be used for this type of measurements. The fluorescence time trace of these measurements is shown in fig. 8.

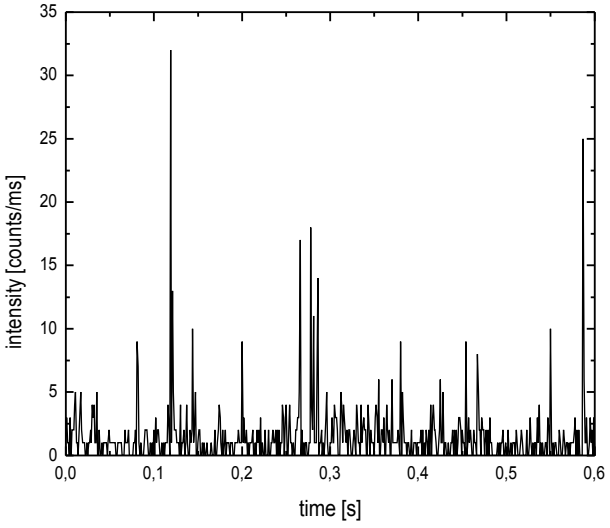

Fig. 8: Fluorescence time trace of single Atto655 molecules diffusing through the confocal detection volume.

Fluorescence bursts from single Atto655 molecules which are diffusing statistically through the confocal investigation volume can be clearly seen. The typical count rate is about a factor of two lower compared with the SPCM-AQR SPADS, which is consistent with the theoretical data that shows about 30% quantum efficiency for the PDM 50CT compared to about 62% of the SPCM-AQR SPADs at this wavelength. However, the signal rate is still high enough to calculate the fluorescence correlation curve, as shown in fig. 9.

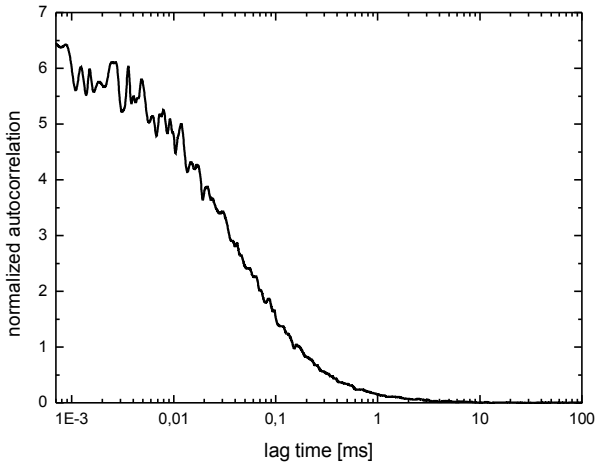

Fig. 9: FCS curve calculated from the MCS trace in fig. 8 for a 100 s measurement.

Summary

The MPD PDM 50CT SPAD has a very good timing response, which is comparable to MCP-PMTs. One striking feature compared to the currently used SPADs of the SPCM-AQR Series from Perkin Elmer is the independence of the temporal position and the full width at half maximum of the Instrument Response Function (IRF) from the signal count rate, which is of high importance for single molecule applications and Fluorescence Lifetime Imaging (FLIM). The PDM 50CT is by far more sensitive in the blue spectral region compared to the SPCM-AQR SPADs, whereas the spectral sensitivity in the red is lower, but still high enough to allow for single molecule measurements.

The SPAD has been found to be very robust – even exposure to daylight during operation did not do any damage. The integration of these SPADs into the MicroTime 200 is not problematic at all, especially as the position of the focus on the active area is relatively uncritical compared to the SPCM-AQR SPADs. First measurements showed, that the PDM 50CT can very well be used for single molecule measurements. Besides single molecule applications, the SPADs can also be used to replace expensive MCP-PMTs for fast timing applications, e.g. for laser pulse characterisation. The active area of the SPAD is, however, too small for ultra sensitive detection of large volume type samples (e.g. cuvettes) in spectrometer applications.

Acknowledgement

We would like to thank Micro Photon Devices for giving us the opportunity to test the PDM 50 CT.

Further reading

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[3] Wahl, M., Koberling, F., Patting, M., Rahn, H., Erdmann, R. , Curr. Pharm. Biotech., Vol. 5, p. 299-308 (2004)
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[6] see *http://www.hamamatsu.com*

[7] Wahl M., Erdmann R., Lauritsen K., Rahn H.-J. , Proceedings of SPIE, Vol.3259, p.173-178 (1998)
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