COMPARISON OF TWO CAMERAS BASED ON SINGLE PHOTON AVALANCHE DIODES (SPADS) FOR FLUORESCENCE LIFETIME IMAGING APPLICATION WITH PICOSECOND RESOLUTION

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1. Introduction.

We report on a comparative study of two time-resolved cameras based on digital Single Photon Avalanche Diodes (SPADs) for Fluorescence Lifetime Imaging applications. In contrast to standard imagers such as cameras, SPAD imagers provide intensity as well as direct access to the fluorescence decay temporal profile, from which lifetime contrast can be extracted. Intensity images provide physicians with anatomical information to localize cancerous tissue; lifetime maps on the other hand provide metabolic information on tissues and can help detect the presence and location of metastatic cells [1,2,3]. With a timing resolution better than 100ps in a compact setup, the SPAD camera represents an innovative solution to explore subnanosecond fluorescence mechanisms.

2. Camera based on the SPSD chip.

The proposed camera is the reformulation of a photodetection system called SPSD (Single Photon Synchronous Detection [4]), originally developed for time-resolved 3D imaging, for fluorescence lifetime imaging. The device consists of a 60x48 array of pixels with a pitch of 85µm, each pixel comprising a SPAD, two counters, and a switch. A complete test setup integrating a working camera driven by an FPGA was developed. High timing resolution is possible by means of a gating scheme controlled by delay lines with a resolution of 10ps, as shown in Figure 1. The SPSD camera offers a parallel multi-channel analysis of single-photon signals with a time resolution of 100ps. The main features of the camera are described in Table 1.

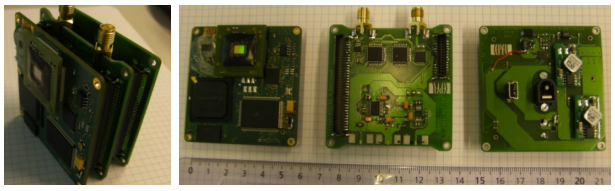

Figure 1: SPSD camera comprising (from left to right) the SPSD chip, an FPGA control board, a delay lines board, and a power supply board.

3. Camera based on the SwissSPAD chip.

a) Chip description

SwissSPAD is an image sensor using the largest existing array of individually addressable SPAD pixels. It is shown in Figure 2. A pixel consists of a SPAD, a 1-bit memory and the read-out circuit. The sensors resolution is 512x128 pixels at a pitch of 24µm. An electronic shutter enables precise control of the photon sensitive time window. Integration times shorter than 5ns with accurate start and stop definitions have been demonstrated. The readout of a 1-bit image as 128 parallel line readouts requires 6.4µs. An FPGA is used to control the chip and to interface it with a computer. Its main features are described in Table 1 in comparison with those of the SPSD camera.

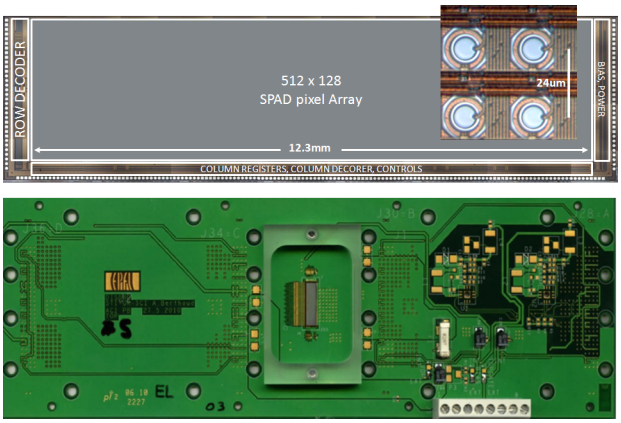

Figure 2: Top: Photomicrograph of the SwissSPAD sensor chip with pixel details in the inset. Bottom: Photograph of the chip-on-board style PCB. The chip, fabricated in 0.35µm CMOS technology, has an area of 13.5x3.5mm^2 and it is fully buttable with an identical chip along the long edge to achieve an array of 512x256 pixels with a gap of 144µm.

Table 1: Performance summary for the two image sensors. All parameters were measured at room temperature.

		SPSD	SwissSPAD
Pixel	Chip size [mm^2]	6.5x5.5	13.5x3.5
	Nb. of pixels	60x48	512x128
	Pixel pitch [µm]	85	24
	Fill factor [%]	0.8	10
	Photon Detection Probability @835nm [%]	2	2
	Dead time [ns]	40	100
	DCR @ room temperature [Hz]	245	206
	CMOS process [µm]	0.35	0.35
System	Gating duration [ns]	5	5
	Gating Step [ps]	10	20
	Matrix Readout Time [µs]	10.8	6.4
	Exposure time ratio [%]	100	25 - 50

a) Timing characterization

The most important element to characterize in the SwissSPAD array is the gating circuit. It is composed of the transistor switches in all the pixels controlled by global signals distributed across the sensor array. In the case of SwissSPAD the signals are physically routed from the bottom to the top and the individual columns are driven from a balanced driver tree at the bottom. This approach was chosen to reduce the signal skew across the

array as much as possible.

Figure 3 shows the distribution of one reference edge of the gate obtained when imaging the pulses of a pico-second laser. The signals are spread over approximately 500ps and from the spatial distribution we can see that there is a significant systematic portion delaying the signal from bottom to top along with a random portion introduced by fabrication non linearities. Calibration can be done to reduce the spread and eliminate the systematic part.

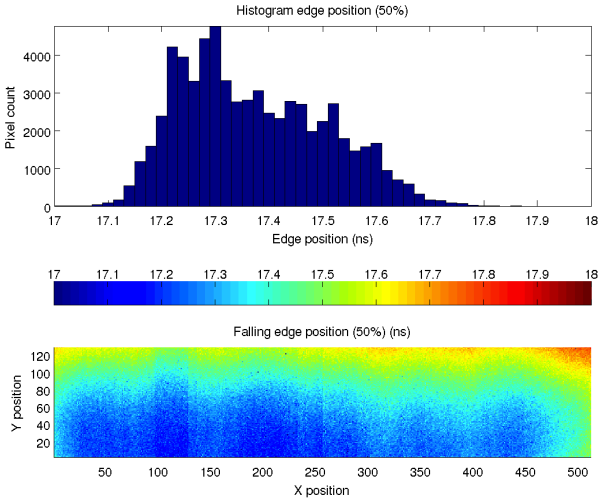

Figure 3]: Timing distribution of the gating signals across the SwissSPAD array. The large majority of pixels are covered with a window of 500ps. The spatial distribution shows the systematic skew introduced through our specific architecture. Calibration can eliminate this part of the skew.

4. Test setup.

A first laboratory test setup was developed to characterize Indocyanine Green (ICG), a fluorescent marker approved by the Food and Drug Administration in the USA. The setup comprises a laser, which emits light at 790nm with a pulse width of 55ps and at a rate of 100MHz. The optical setup permits to direct the laser light towards the sample and to drive the fluorescence emission at 835nm towards the camera. Two wavelength filters and a dichroic mirror allow the camera to detect only the fluorescence photons. Two optical configurations were used. The first one, shown in Figure 4, is a point detection configuration. It allows detecting a larger number of photons as the laser light is focused onto a small point of the target.

In the second configuration – an imaging configuration – plano-convex lenses are used to obtain an image of the samples on the image plane without magnification (see Figure 5).

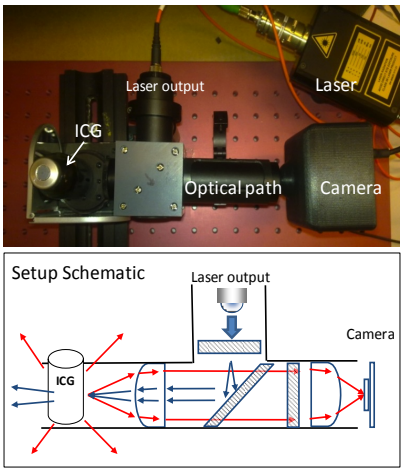

Figure 4: Picture (top) and schematic (bottom) of the Point detection setup.

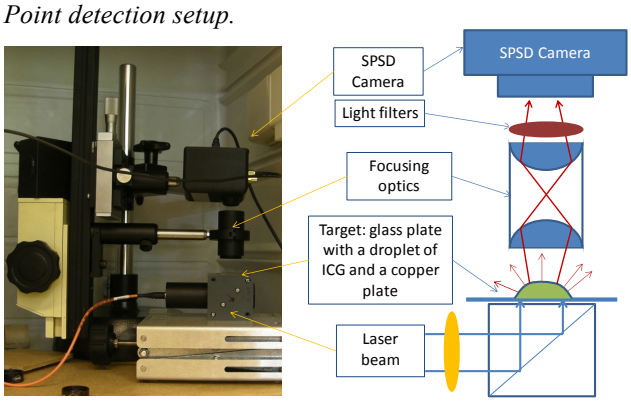

Figure 5: Picture (left) and schematic (right) of the imaging setup.

The samples analyzed are solutions of ICG diluted in water, milk (3.5% fat) and a 1% intralipid solution. ICG was diluted from 2μM up to 80μM, so as to be in the same conditions as [5] for a quantitative comparison. A thin micromachined copper plate (shown on the top left of Figure 8) acts as a mask for the laser light and allows controlling the imaging quality. The cameras acquire the time resolved data, which is analyzed offline using Matlab routines.

5. Results with the point detection setup:

a) Sensitivity

Intensity pictures were taken, both with the SwissSPAD and the SPSD cameras. The samples studied are ICG in milk, at concentrations between 10μM and 40μM. Figure 6 shows for each detection system the projection of the point on the camera. Note that as milk is a very diffusive medium, the fluorescence photons do not come from a point, but from a spot.

The high dark count rate from the SPSD camera is due to the high temperature of 60ºC generated by the delay lines. The relatively low number of detected photons is explained by the quantum yield of ICG (~1%), the fraction of fluorescence photons reaching the optical setup (~0.6%), the fill factor of the chips and the quantum efficiency in the near infrared (~2%).

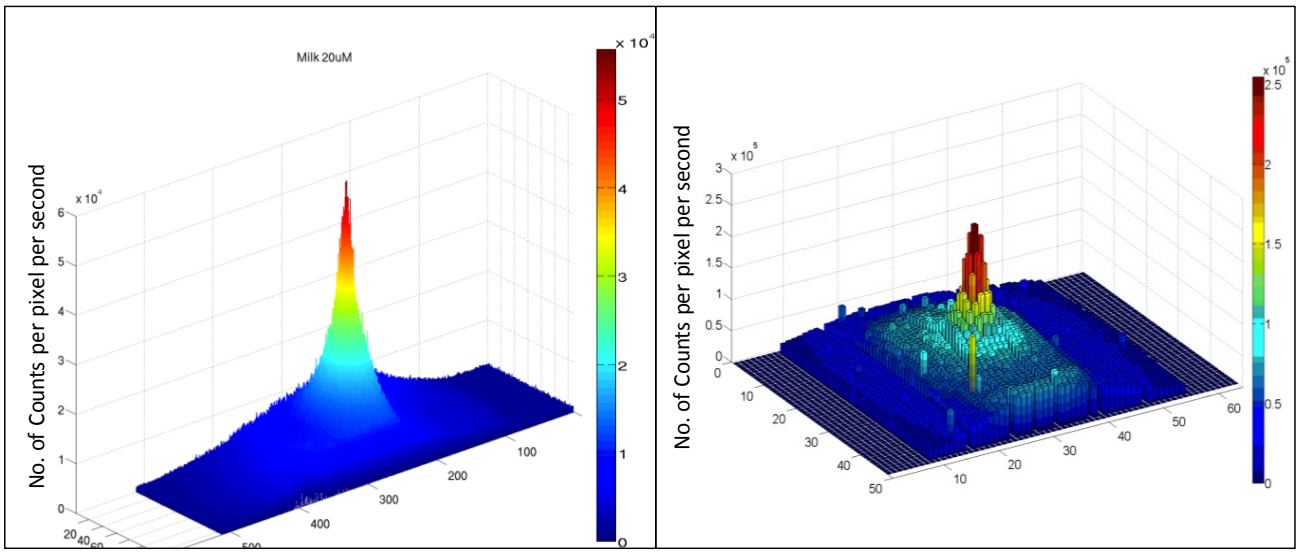

Figure 6: Intensity images of the SwissSPAD camera (left) and of the SPSD camera (right). The point detection setup is used. The target is ICG in milk, at a concentration of 20µM.

The SwissSPAD and SPSD cameras see a total of 11.2×10^7 and 5.5×10^7 photons per second in the spot area, respectively. This ratio of two is explained as follows: on one hand, with a fill factor ten times higher, the SwissSPAD camera will see a larger number of photons. On the other hand, its gate was only active 25% of the time whereas the SPSD camera always counts photons, using its two counters. Therefore the ratio between the numbers of photons observed is of 2.5, which is in good agreement with the results shown here.

b) Timing performance

The fluorescence decay time constant of the ICG signal is then calculated. Figure 7 shows, for the SPSD camera, the time constant extracted as a function of the ICG concentration in the three different media. Similar measurements are shown with the SwissSPAD camera with ICG in milk. The results obtained with both cameras are in good agreement with results of ref. [5].

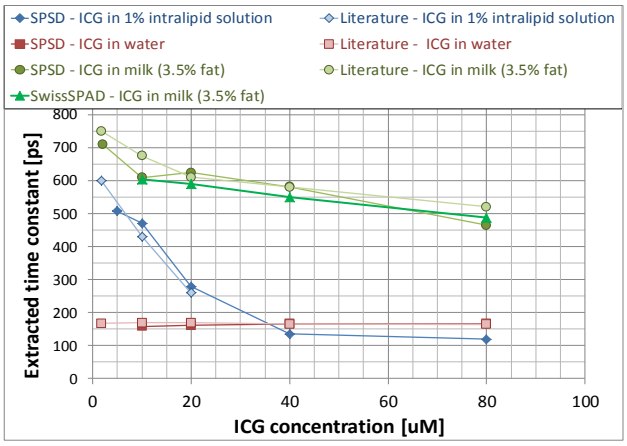

Figure 7: Extracted fluorescence time constant vs. ICG concentration in various media. Results obtained from both SPSD and SwissSPAD cameras. Good agreement between measurements and literature [5] is observed.

Additional measurements were carried out with the SwissSPAD camera mounted on a fluorescence microscopy setup using a 532nm pulsed laser and a set of visible wavelength emitting dyes. The lifetimes are fitted by convolution of a single exponential with the instrument response function (IRF), measured with a scattering sample (Ludox), as shown in Figure 8. These measurements show reasonable agreement with published data [6], as shown in Table 2, considering the high dye concentration used during the experiments.

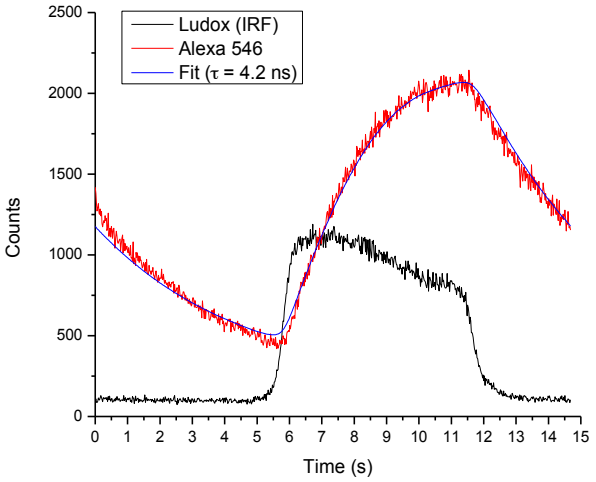

Figure 8: Sample data recorded by SwissSPAD. The curves show the measured signal collected over a 5ns sliding integration window. The response of the fluorophore (red) is the convolution of the IRF (black) and a single exponential decay with the characteristic lifetime of the fluorophore. The lifetime is extracted through curve fitting (blue).

Table 2: Fitted lifetime of different fluorescent dyes obtained with the SwissSPAD camera and comparison with reference data from [6].

Fluorescent dye	Reference lifetime from [6] [ns]	lifetime from SwissSPAD [ns]
Rhodamine 6G	4.08	3.8
CY3B	2.8	2.5
Alexa Fluor 546	4.0	4.2

6. **Results with the imaging setup**:

An intensity picture obtained with the imaging setup and the SPSD camera is shown in Figure 9. The sample studied here is ICG in milk with a concentration of 20µM. For an integration time of 1 second, between 20,000 and 40,000 photons are detected on each pixel, with a noise of 3,000 counts per pixel. The lifetime calculated on each pixel is shown in Figure 10. The extracted time constants feature an average of 610ps with a standard deviation of 60ps, which is in good agreement with the results shown in Figure 7.

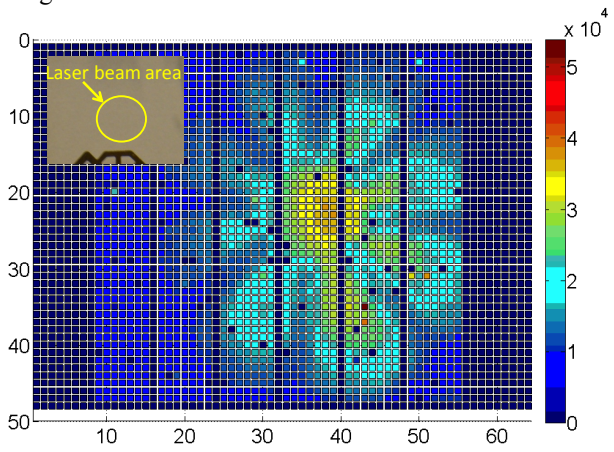

Figure 9: Fluorescence intensity image obtained with the Imaging setup and the SPSD camera. The mask used is shown in the top left corner.

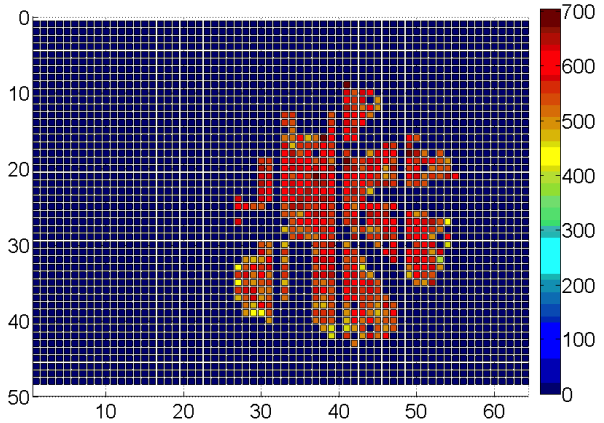

Figure 10: Corresponding Fluorescence lifetime image obtained with the Imaging setup and the SPSD camera. Good homogeneity is seen over the pixels, with a standard deviation of 60ps.

7. **Conclusion**:

Results obtained from the SwissSPAD and the SPSD cameras are shown in fluorescence lifetime imaging applications. These measurements were used in preliminary studies to analyze various fluorophores in different media and at different concentrations.
The intensity figures obtained show the possibility to acquire images with good signal-to-noise ratio. Thanks to the SPAD technology, these cameras are able to extract fluorescence time constants as short as 170ps, with a time resolution better than 100ps.
They represent very promising tools for the biological community to study sub-nanoseconds fluorescence phenomena in a compact and cost effective implementation.

Acknowledgements

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