SPC-QC-104: Precision FLIM and Fast FLIM in One

Abstract: The bh SPC-QC-104 TCSPC/FLIM module has three parallel photon timing channels plus one reference channel. With an IRF width of <38 ps FWHM, <17 ps RMS timing jitter, 4 ps minimum time-channel width and the ability to run both precision FLIM and 'Express' FLIM, the SPC-QC is an excellent choice for FLIM with confocal and multiphoton laser scanning systems. The performance in typical applications will be demonstrated in this application note.

General Features

The SPC-QC-104 TCSPC / FLIM module has three parallel TCSPC / FLIM channels or four absolute time-tagging channels on a single PCI-express board. Different than the SPC-150 and SPC-180 modules, the SPC-QC uses a direct time-to digital (TDC) principle for photon timing. Compared with the TAC/ADC principle used in the SPC modules, the TDC principle has the advantage of lower dead time, higher peak count rate, and lower power consumption. Although the TDC does not reach the time resolution of the TAC/ADC principle the resolution is absolutely sufficient for most of the typical FLIM applications. The electrical IRF width is less than 38 ps FWHM; the timing jitter is less than 17 ps RMS. The timing stability over 10 minutes is better than 5 ps RMS, including a bh BDS-SM ps diode laser and a bh HPM-100-06 detector. The peak count rate is on the order of 120 MHz per channel [3]. In terms of photon recording, the SPC-QC-104 module uses the same multi-dimensional recording principles as the SPC modules [1, 2]. These features make the SPC-QC-104 an excellent choice for FLIM recording in laser scanning systems. A FLIM image recorded at a resolution of 512 x 512 pixels and 256 time channels is shown Fig. 1, left, a decay curve in a 3x3 pixel area in Fig. 1, right.

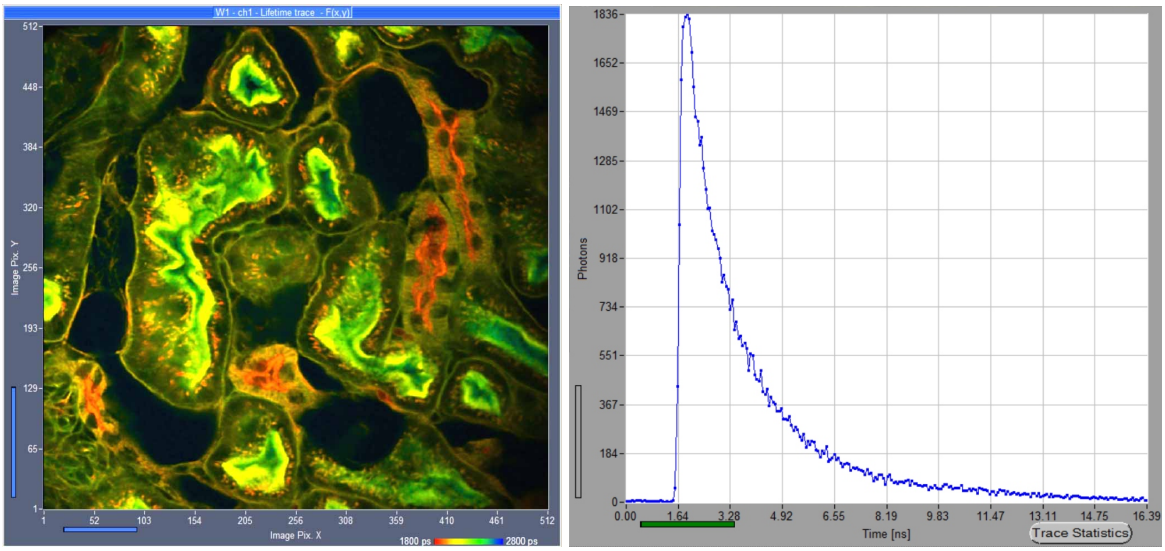

Fig. 1: FLIM image recorded with the SPC-QC-104. Right: Decay curve in 3x3 pixel area. Invitrogen Mouse kidney sample. DCS-120 MP multiphoton FLIM system, 512 x 512 pixels, 256 time channels. Online-FLIM and Curve display functions of SPCM [1].

High-Resolution Images

In the past few years, bh have more and more passed to FLIM data formats with higher numbers of pixels and time channels. Higher number of time channels does not cause any degradation in lifetime accuracy [6, 7] but provides better options to extract fast decay components, higher number of pixels yields images with better spatial resolution. The resulting decrease in the number of photons per pixel can be compensated by the overlapping binning function of SPCImage [6, 7]. An example of a high-resolution dual-channel FLIM recording is shown in Fig. 2 and Fig. 3.

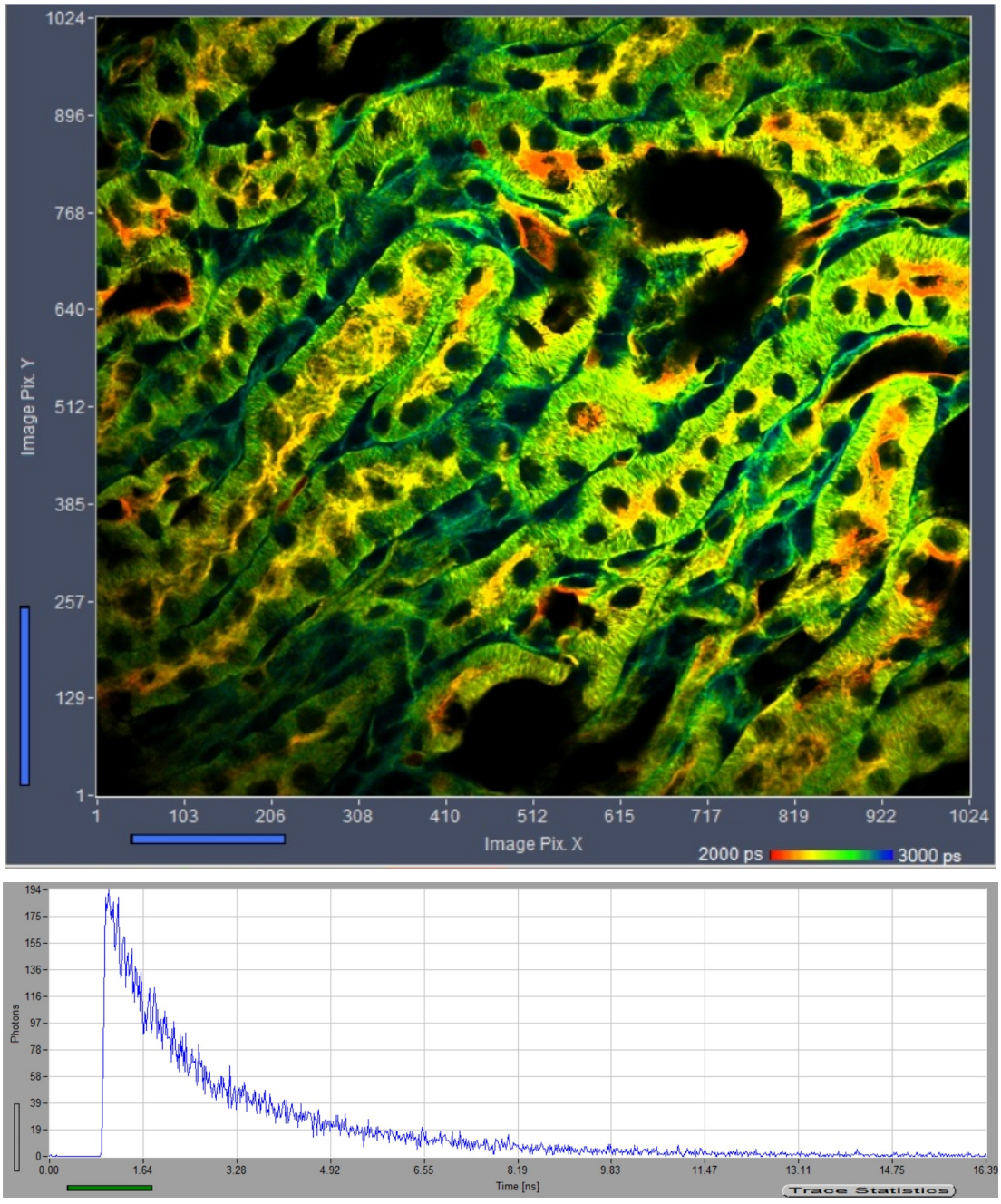

Fig. 2: Mouse kidney sample, DCS-120 MP, FLIM image from channel <510 nm, 1024 x 1024 pixels, 1024 time channels. Bottom: Decay curve over an area of 5x5 pixels.

The images show a mouse kidney section (Invitrogen) recorded with 1024 x 1024 pixels and 1024 time channels in the two spectral channels of a DCS-120 MP multiphoton FLIM system [5]. Decay curves from a 5x5 pixel area are shown below the images.

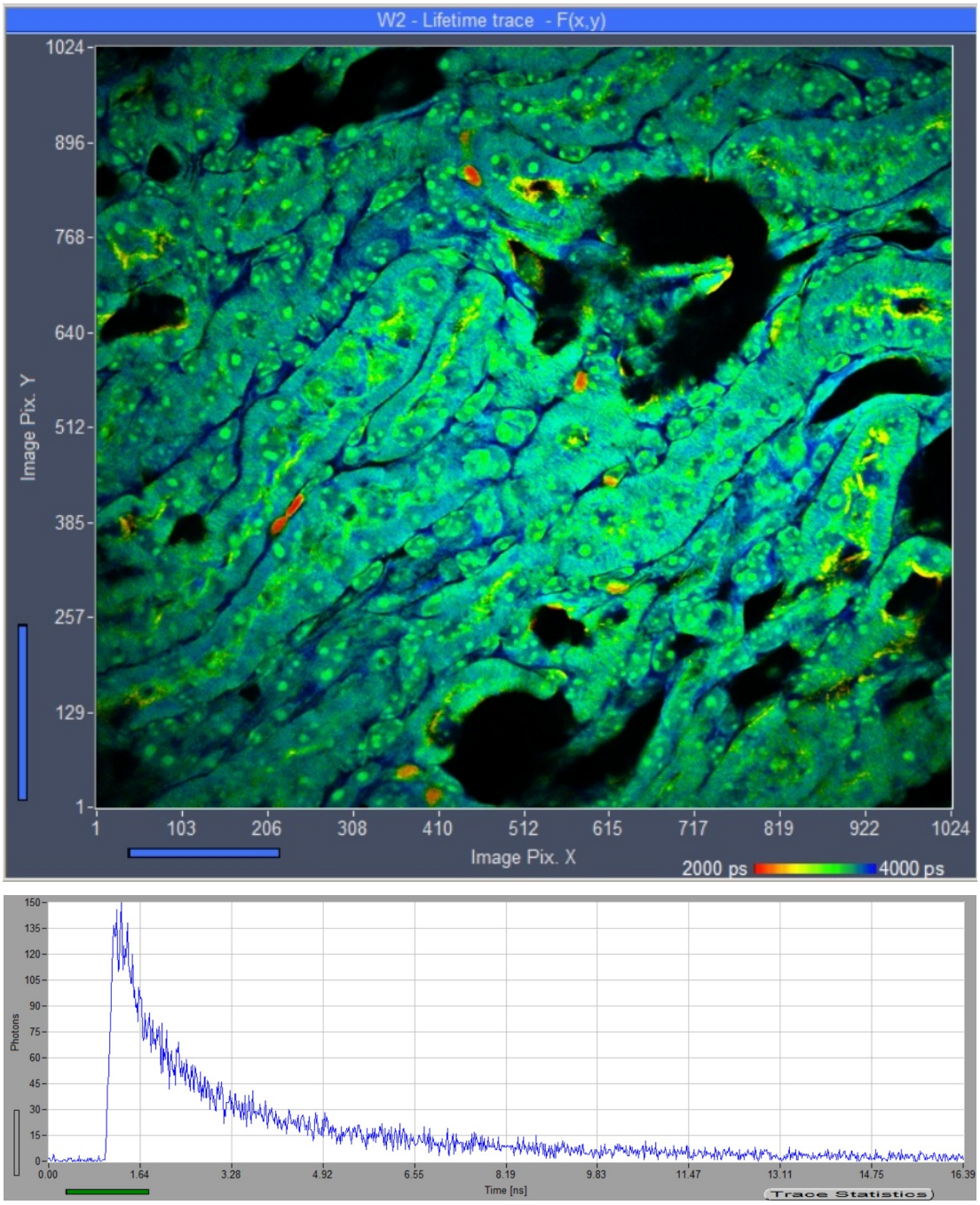

Fig. 3: Mouse kidney sample, DCS-120 MP system, FLIM image from channel >510 nm, 1024 x 1024 pixels, 1024 time channels. Bottom: Decay curve over an area of 5x5 pixels

Analysis with SPCImage NG

SPC-QC FLIM data files are analysed by bh SPCImage NG software the same way as SPC data [7]. Large numbers of pixels and time channels are no problem for SPCImage NG. The analysis algorithms run on a GPU, reducing the analysis time to a few seconds even for the largest images. Single, double and triple-exponential decay models are available. Incomplete decay within the signal period is taken into account by an 'incomplete-decay' option, weighting problems at low photon counts are avoided by a maximum-likelihood (MLE) algorithm [7]. SPCImage analysis of the data of Fig. 2 is demonstrated in Fig. 4 through Fig. 6. A fit with a triple-exponential incomplete-decay model in combination with the synthetic IRF of SPCImage was used. Fig. 4 shows the entire SPCImage panel with an image of the amplitude-weighted lifetime, tm, a decay curve at the cursor position, and the residuals of the fit. The decay data are clean, and the smooth residuals indicate a high reliability of the fit. Images of the lifetimes of the decay components, t1, t2, t3, and of the amplitudes of the components, a1, a2, a3 are shown in Fig. 5 and Fig. 6. As can be seen from these images, triple-exponential decay parameters are extracted from the decay data at a remarkably high accuracy.

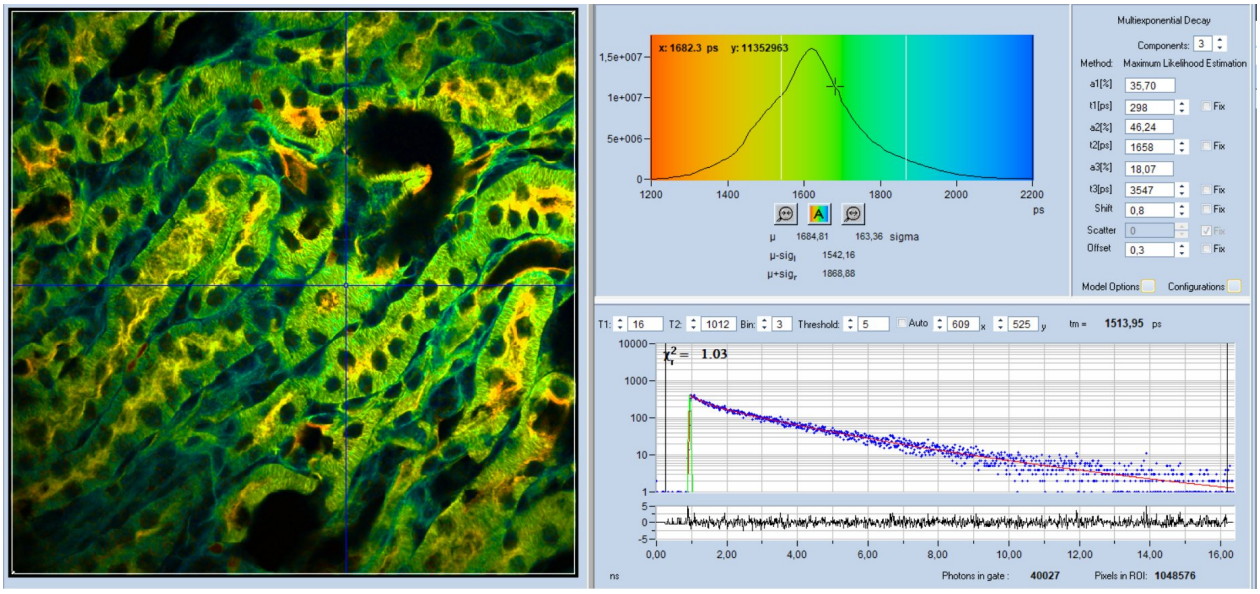

Fig. 4: Data from Fig. 2, analysis by SPCImage NG. Triple-exponential fit, image of amplitude-weighted lifetime, tm.

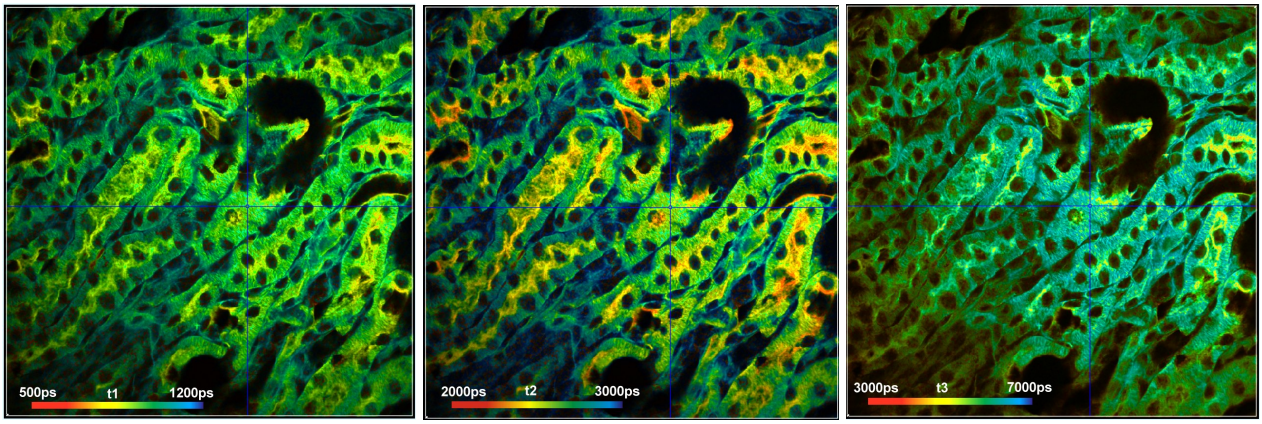

Fig. 5: Images of lifetimes of decay components, t1, t2, t3

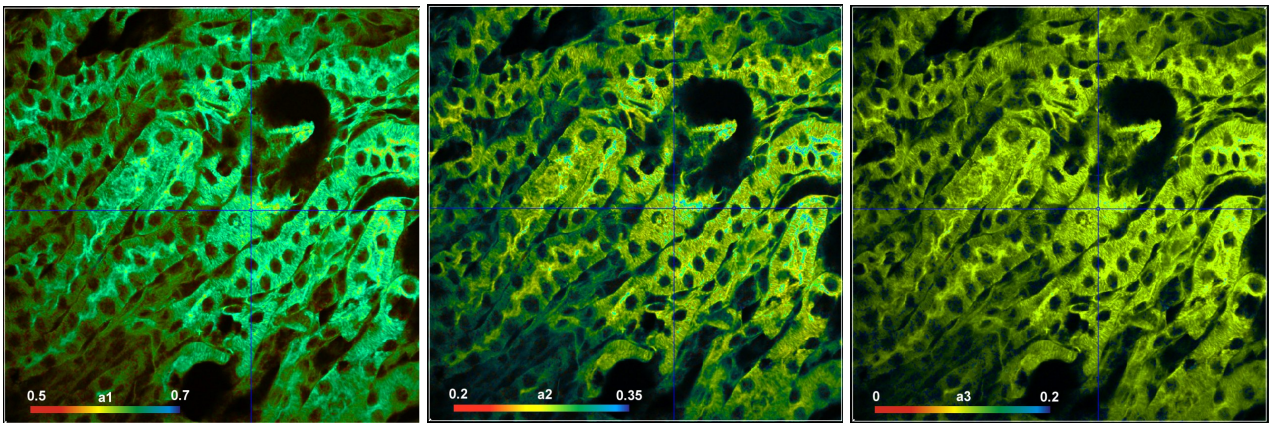

Fig. 6: Images of amplitudes of decay components, a1, a2, a3

Express FLIM: As Fast as the Scanner Can Go

Due to its short dead time the SPC-QC-104 is able to record FLIM at high count rates and, consequently, short acquisition time. An example is shown in Fig. 7. An autofluorescence lifetime image of Enchytraeus albidus (a little worm) was recorded at 8 MHz count rate and within an acquisition time of one second. The image was recorded in the standard 'FIFO Imaging' mode of the SPCM software. The image format is 512x512 pixels with 1024 time channels per pixel.

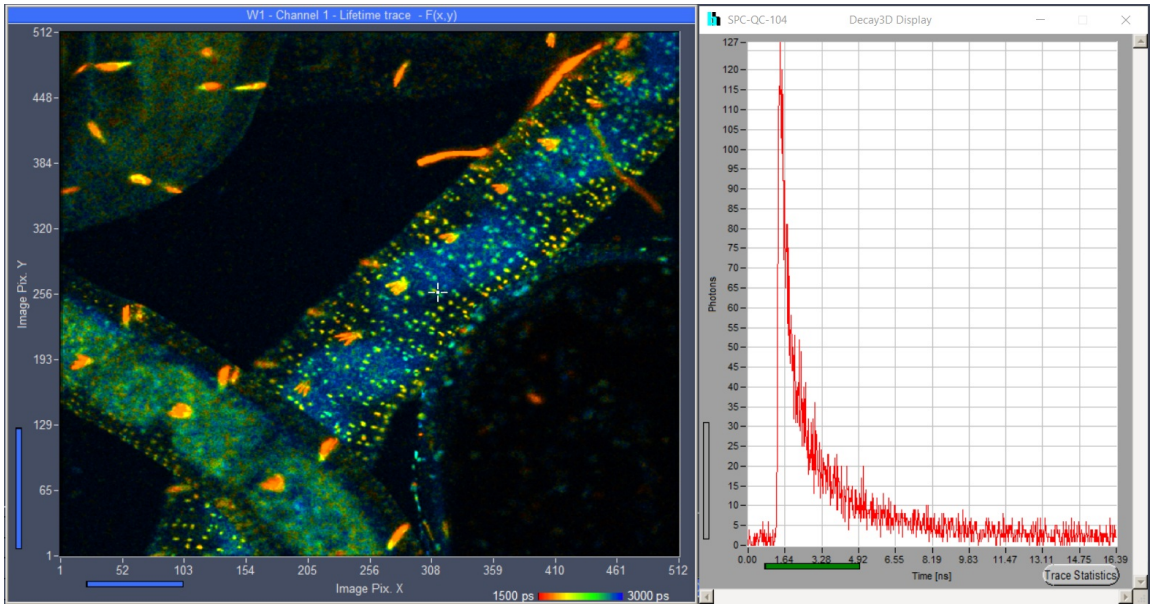

Fig. 7: Lifetime image taken from a live Enchytraeus albidus. Autofluorescence, 1 second acquisition time at 8 MHz average count rate, DCS-120 system with SPC-QC-104, Zeiss Axio Observer microscope, x10 objective lens. Image area about 1 mm x 1 mm. Online FLIM with SPCM software.

Recording an image as the one shown above in a short period of time requires an enormous data amount of data to be transferred from the TCSPC module to the computer. The requirements to the data transfer rate increase if not only one channel but all three channels of the SPC-QC are operated at high count rate. Possible bus saturation then sets a limit to the recordable photon rate.

A solution to the data transfer problem is the bh 'Express FLIM' technique [1, 4]. Express FLIM does not transfer the data into the computer photon by photon. Instead, the hardware of the SPC-QC module combines the information of all photons within a given pixel into a just two numbers. One is the first moment of the decay curve, the other the number of photons within the pixel. Both numbers are transferred to the computer at the end of each pixel [1]. Even for very fast scanning, the required data transfer rate can easily be achieved. The result is a lifetime image that contains intensity-weighted lifetime values in the pixels. In practice, the image rate obtained by Express FLIM is only limited by the frame rate of the scanner. Four images from a sequence of 200-ms recordings (5 frames per second) are shown in Fig. 8.

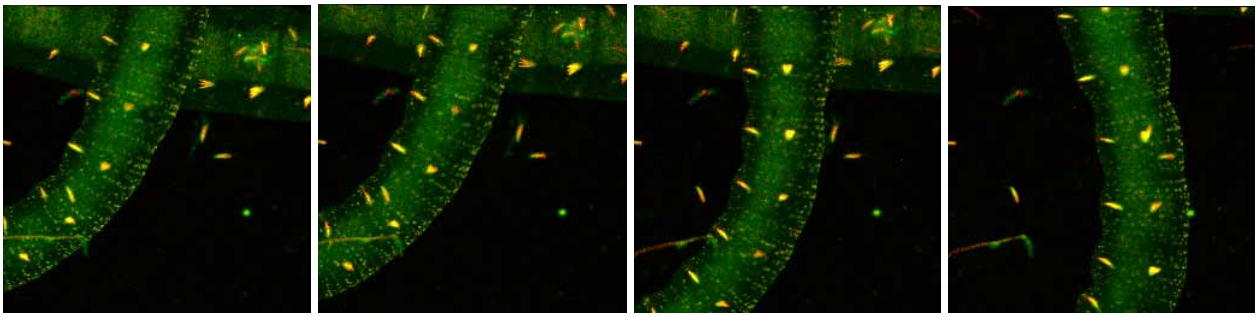

Fig. 8: Express-FLIM of a live Enchytraeus albidus. Autofluorescence, four images from a 5-frames/second sequence. DCS-120 system with SPC-QC-104. Photon Rate about $10 \cdot 10^6$ s^{-1}.

Although the speed and the image quality of Express FLIM is impressive the real charm of the technique is that you *don't have to use it*. How the SPC-QC behaves depends on the software you use it with. Start Express FLIM and you have a fast FLIM system that delivers fast image sequences as shown in Fig. 8. Start SPCM and the SPC-QC will perform as a precision FLIM module, with high temporal resolution and full fluorescence decay data in the individual pixels as shown in Fig. 7.

Comparison with SPC-150 and SPC-180 Modules

A comparison of the SPC-QC-104 with the SPC-150 and SPC-180 modules in FLIM applications is given below.

	SPC-QC-104	**SPC-150NX, -180NX**
FLIM channels on one board	3	1
IRF width, FWHM,	38 ps	3 ps
IRF width, with HPM-100-06 detector	42 ps	19 ps with HPM, 5 ps with SSPDs
Min time-channel width	4 ps	0.4 ps
Peak count rate	120 MHz	10 MHz
Timing principle	TDC	TAC / ADC
Start-Stop sequence	non-reversed start-stop	reversed start-stop
Max image size, pixels	2048 x 2048	2048 x 2048
Precision FLIM	yes	yes
Express FLIM	yes	-
Applications	Standard FLIM	Standard FLIM
	FLIM with Exogenous Fluorophores	FLIM with Exogenous Fluorophores
	FRET FLIM	FRET FLIM
	-	Label-free imaging
	-	Metabolic imaging
	-	Ultra-short lifetimes

As can be seen from the table above, the modules differ mostly in the number of parallel recording channels, the time resolution, the peak count rate, the ability to run Express FLIM, and the start-stop mode. The three channels of the SPC-QC make it a convenient solution for Laser scanning systems with several detection channels, such as the DCS-120 MP, the DCS-120 confocal, and the DCS-120 MACRO, as well as the Zeiss LSM 980 and the Leica SP and Stellaris systems. The SPC modules, on the other hand, have an advantage in time resolution. The superior time resolution of the SPC modules mainly pays off in applications like label-free imaging and metabolic FLIM where extremely fast decay processes are involved. These are better resolved with the SPC modules, especially when they are part of multi-exponential decay functions. On the other hand, the high peak count rate and the ability to run Express FLIM are a plus for the SPC-QC. However, these features should not be over-estimated. The vast majority of FLIM samples do not deliver count rates to run Express FLIM efficiently. Even the peak count rates are well in the range of the SPC modules [1]. The non-reversed start-stop principle can be a benefit when it comes to recording FLIM with fluorophores of long lifetime. Long lifetimes are encountered mainly in semiconductor research, where the lifetimes can be in the range of several 100 ns. Non-reversed start stop then makes a switchable delay in the stop channel unnecessary, simplifying system setup and timing parameter setting.

Summary

The SPC-QC is a cost-efficient and easy-to-use solution for a large number of FLIM applications. The main advantages are the availability of three FLIM channels on one board, and the fact the modules can run both precision FLIM and Express FLIM.

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