Lifetime-Intensity Mode Delivers Better FLIM Images

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Abstract: At count rates approaching the reciprocal dead time of the TCSPC electronics the photon number in the pixels of a FLIM image becomes a nonlinear function of the intensity. The images are therefore losing contrast in bright regions, whereas the decay data in the individual pixels remain correct. To improve the image quality at high count rates we have implemented a counter in parallel with the TCSPC timing electronics. The counter delivers pixel photon numbers with virtually no counting loss. A new 'Lifetime/Intensity' mode of bh's SPCM software builds up FLIM images by using pixel intensities from the parallel counter and pixel decay data from the timing electronics. These images show substantially improved contrast at high count rates. The mode is available for the SPC-160 and the new SPC-180 TCSPC/FLIM modules.

TCSPC Intensity Scale at High Count Rate

TCSPC FLIM images taken at high count rates often look unsightly, with less than ideal contrast and apparent loss in highlight detail. The source of the effect is not the 'pile-up' effect, as commonly believed. Pile-up would change the recorded lifetimes to lower values. However, a decrease of the recorded lifetime is not found in the data. This is understandable: The error in the recorded lifetime remains less than 5% up to a count rate of 20% of the excitation pulse frequency [1, 2]. That means, for 80 MHz laser repetition rate count rates of more than 10 MHz can be used. Count rates that high are rarely encountered in FLIM measurements.

It turns out that the reason of poor contrast at high count rate is counting loss by the dead time of the TCSPC process. Counting loss causes a nonlinearity in the intensity scale [1, 2]. The function of the count number over intensity flattens out at high intensities, and eventually saturates. The effect is a loss in contrast in the bright parts of the image. This is exactly what is observed.

For most FLIM applications the nonlinearity of the intensity scale is a purely aesthetic problem: Information is derived from the shape of the decay curves, not from the intensities in the pixels. Instrumental solutions which sacrifice time resolution or photon efficiency for low dead time are therefore not appropriate [4]. The bh FASTAC FLIM system [5] reaches extremely low dead time without loss in time resolution. However, the technical effort is high, and not normally justified to solve a purely aesthetic problem.

A simple solution to the intensity-nonlinearity problem has been implemented in the bh SPC-160 and, recently, in the SPC-180 TCSPC modules [6]. These modules got a parallel counter implemented that bypasses the TCSPC timing electronics. It counts the pulses directly at the output of the detector CFD, and puts the counter result for every pixel in the pixel clock entry in the parameter-tag data. The dead time of this counter is negligible - it is mainly determined by the detector and CFD dead time. This is about 5 ns. The FLIM images are built up by using the counter results as pixel intensities, and the data from the TCSPC timing electronics as pixel decay curves. The new FLIM mode is available for the SPC-160 and the new SPC-180 family TCSPC modules.

Example

An example of the image improvement is shown in Fig. 1. The images were recorded with a DCS-120 Confocal FLIM system [3] using SPC-180NX TCSPC modules. The sample was a stained mouse kidney section (Invitrogen F24630). The excitation wavelength was 480 nm, the detection wavelength interval 500 to 680 nm. The images were recorded with 512 x 512 pixels, and 1024 time channels per pixel. The recorded count rate averaged over the image was 5.6 MHz. The image on the left was created by the new Lifetime/Intensity mode, the image on the right by the traditional FLIM mode. In both cases, the online-lifetime display function of the SPCM software [1, 7] was used.

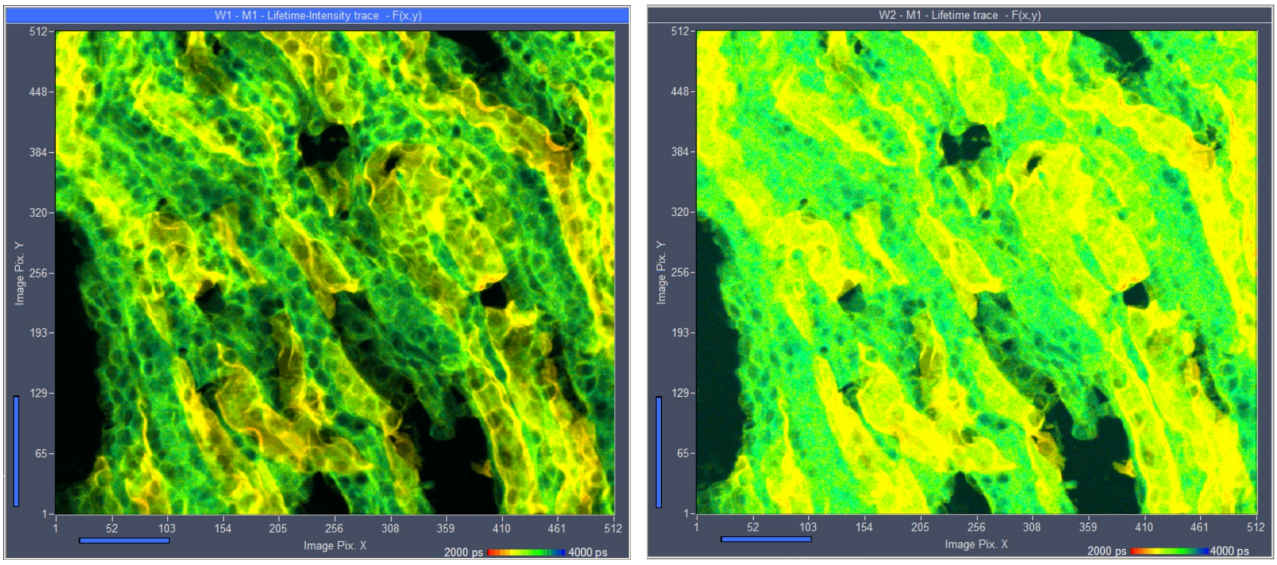

Fig. 1: FLIM images recorded from an Invitrogen F24630 Mouse kidney section. Left: New Lifetime/Intensity mode. Right: Traditional FLIM mode. 512 x 512 pixels, 1024 time channels, online-lifetime display function of SPCM software. Lifetime scale 2000ps to 4000ps. Average (recorded) count rate 5.5 MHz.

Implementation in the SPCM Software and Parameter Setup

The Lifetime/Intensity mode is available in the SPCM data acquisition software, Version 9.86 of June 8, 2021 or later. The implementation in the user interface and the parameter setup are shown in Fig. 2 and Fig. 3. In the 'System parameters', click on 'Configure' and enable 'Intensity Image / Build Image'. This enables the parallel counter, puts the pixel intensities in the photon data stream, and instructs the software to build up an array of pixel intensities.

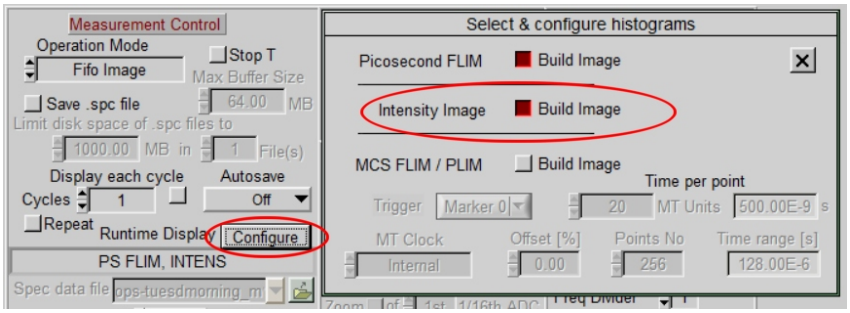

Fig. 2: System parameters for intensity imaging via the parallel counter channel. SPCM builds up an array of the pixel intensities recorded by the parallel counter.

In the 3D Trace parameters (accessible via 'Parameters' or right mouse click in an image window), select 'Lifet+I' (Lifetime + Intensity) as a data type, see Fig. 3. You can select 'Lifet+I' for one channel or for several channels simultaneously. In Fig. 3 it has been set for Display Window 1, Display Window 2 shows a 'normal' lifetime image created by the online-lifetime function of SPCM [1, 7]. The images for a second TCSPC module, M2, have been turned off. With the settings shown, SPCM displays the two images shown in Fig. 1.

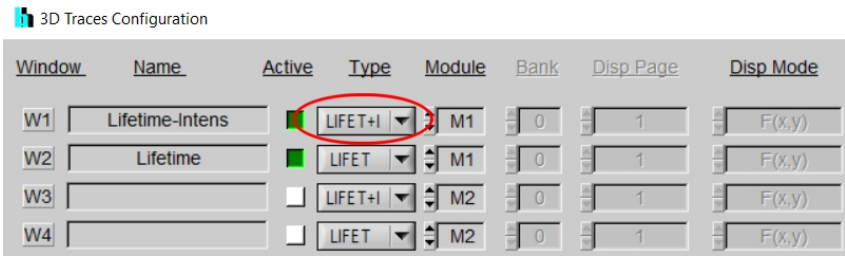

Fig. 3: Enabling the buildup of a Lifetime/Intensity image using the intensity values from the parallel counter

Analysis with SPCImage NG

Analysis of Lifetime/Intensity data is implemented in SPCImage NG, Version 8.4 or later. Data analysis is performed the usual way [1, 8]. Right-click into the SPCM display window of interest, and select 'Send Data to SPCImage', or click into 'Main' and 'Send Data to SPCImage'. SPCImage recognises that the data are Lifetime/Intensity data, and handles them accordingly. That means, the decay curves are taken from the normal FLIM data array, but the intensities come from the array of pixel photon numbers created by the parallel counter channel. To start the data analysis, select an appropriate decay model, and start the procedure via 'Calculate', 'Decay Matrix'. The result for the data of Fig. 1, left, is shown in Fig. 4.

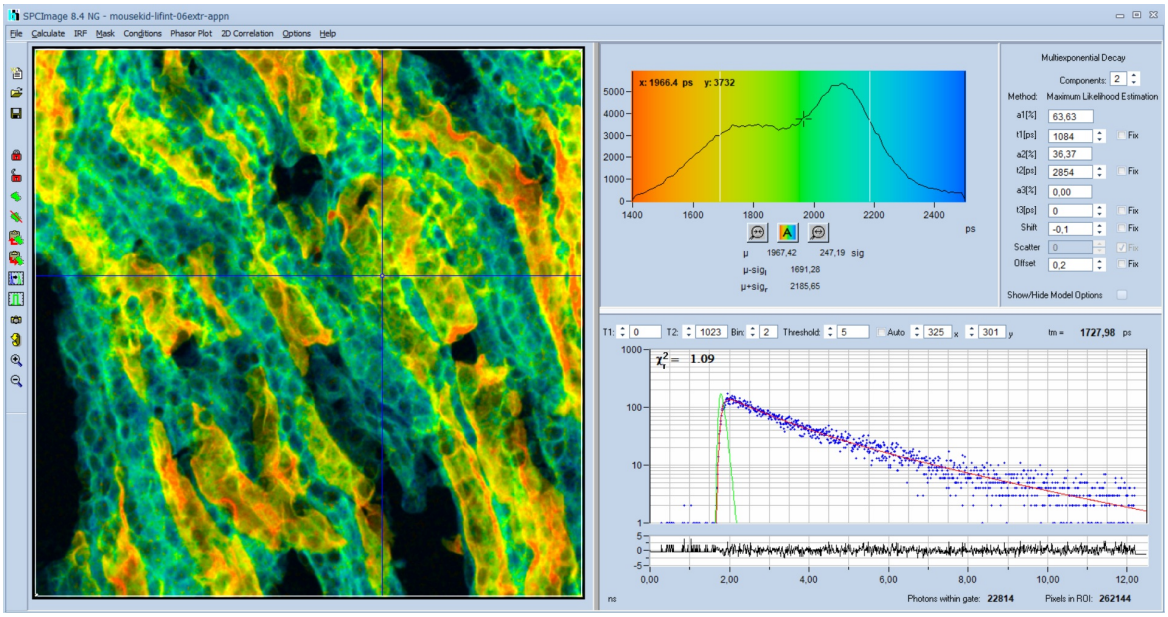

Fig. 4: Analysis of Lifetime / Intensity data by SPCImage NG. Mean lifetime of double-exponential decay, t_m, lifetime range from 1400 ps to 2500 ps.

Summary

The Lifetime/Intensity mode of SPCM, Version 9.86, in combination with the bh SPC-160 and SPC-180 family TCSPC modules records FLIM images at a linear intensity scale up to the saturated count rate of the TCSPC timing electronics. This is achieved by counting the photons in a fast counter in parallel to the TCSPC timing electronics. Pixel intensities derived from the counting results are free of counting loss. The data can be displayed by the 'Lifetime' display function of SPCM. For further analysis, the data can be sent to SPCImage NG the usual way. SPCImage automatically recognises the data as Lifetime/Intensity data and displays them at a linear intensity scale.

References

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