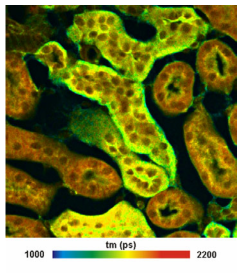
SPC-160 Delivers Linear Intensities in High Count Rate FLIM Applications

At high count rates the photon numbers recorded in TCSPC FLIM are impaired by counting loss effects. The photon numbers in the pixels therefore becomes a nonlinear function of the pixel intensity. Consequently, the images are more and more losing contrast with increasing count rate. In the new SPC-160 TCSPC FLIM modules we therefore implemented a parallel counter channel that delivers pixel photon number with virtually no counting loss. FLIM images built up from lifetimes of the TCSPC data and intensities from the parallel counter show substantially improved contrast compared to conventional FLIM images.



FLIM at High Count Rates

In the last five years, the optical efficiency of FLIM systems has been improved considerably. The optical systems of laser scanning microscopes have become more efficient, better detectors have been introduced [2], and tuneable excitation sources allow the excitation wavelength to be matched to the absorption band of the fluorophores [1]. Consequently, FLIM experiments are performed at increasingly higher count rates. High count rates help keep the acquisition times of megapixel FLIM applications [3] in reasonable limits, and help obtain higher lifetime accuracy in lifetime-transient recordings [1, 6].

Interestingly, the problem of TCSPC FLIM at high count rates is not the pile-up effect, as commonly believed. The error in the recorded lifetime remains less than 5% up to a count rate of 20% of the excitation pulse frequency [1]. That means, for 80 MHz laser repetition rate count rates of more than 10 MHz can be used. More annoying than pile-up is the nonlinearity of the intensity scale by counting loss in the dead time of the photon timing electronics [1]. TCSPC FLIM images recorded at extremely high count rates thus lack intensity contrast. The images appear ‘flat’ and unappealing, see Fig. 1, left (page 2).

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. However, intensities can be important if the fluorescence signals of several fluorophores have to be unmixed from data recorded in several wavelength channels, or if two-dimensional pixel histograms of decay data and intensities are used to separate different fluorescent species.

Parallel Counter Channel

A radical way to avoid intensity nonlinearity is to use a parallel counter channel that does not go through the timing electronics of the TCSPC board. This counter and the required data pathway have been integrated in the new SPC-160 TCSPC modules [4].

The input pulses for the counter come directly from the input constant-fraction discriminator (CFD) of the TCSPC device. The CFDs of the bh TCSPC modules have about 5 ns dead time. With PMTs or hybrid detectors (which have virtually no dead time) the counter delivers intensities unaffected by counting loss. The photon numbers recorded in the parallel counter are transferred into the computer on a pixel-by pixel basis and stored together with the FLIM data from the timing electronics. The SPCImage data analysis [15] builds up the FLIM images by using the fluorescence lifetimes from the TCSPC channel and the intensities from the parallel counter channel. The improvement in image contrast over conventional FLIM data is substantial, compare Fig. 1, left and right.

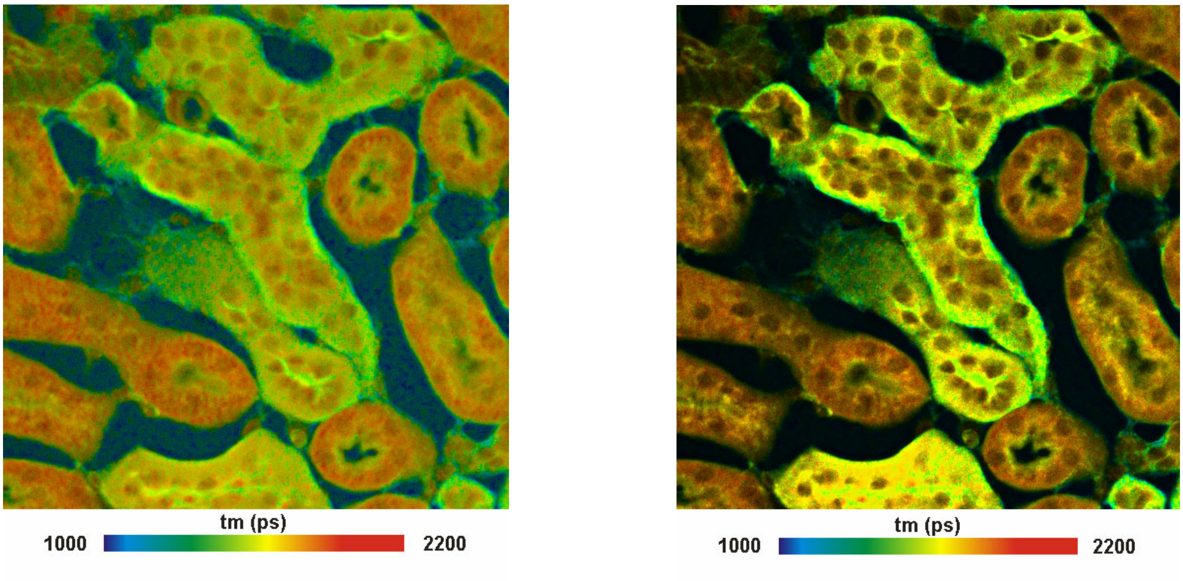


Fig. 1: Mouse kidney sample recorded at an average count rate of 5 MHz. Left: Intensity from TCSPC channel. Right: Intensity from parallel counter channel. bh SPC-160 TCSPC FLIM module [4], DCS-120 confocal scanner [5], SPCImage FLIM data analysis [1, 5]

The detector peak count rates in the bright areas of the images are exceeding 10 MHz. Nevertheless, no differences in the recorded lifetimes can be seen between the two images.

References

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