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How to Avoid the Pile-up Effect in FLIM Measurements

For a definition of the pile-up effect see here: Pile-Up Effect

To avoid pile-up effect the measured count rate should not exceed a certain limit in respect to the laser repetition rate.

- For FLIM measurements we advice a count rate of maximal 10% of the laser repetition rate.

We have performed an example measurement with a fluorophore having a lifetime of 4 ns. At 20 MHz laser repetition rate and 2 MHz detection count rate (10%) and a total count number of 10.000 photons in the TCSPC histogram the following results are obtained:

- the average lifetime decrease is 3%
- the χ^2 value increases from 1.08 to 1.10
- an additional short component lifetime could still not be detected

For most FLIM measurements this relative weak pile up effect is acceptable.

If even higher precision is needed for the lifetime measurement the count rate should be lowered to 1% of the repetition rate of the laser.

Measurement of the Pile Up Limit

- For setting the right laser intensity for FLIM measurements use the **“Test”**-mode and display the image in the **“Image”**-tab.
- For detection of the pile up limit the count rate displayed in the Time Trace below the FLIM image is used.
- A red line indicates the count rate limit which is 10% of the laser repetition rate.
- The **“Time Trace Binning”** should be set to 0.01 ms which equals to 10 µs. The “Number of Points” should be set to 100. In this way, the average count rate is much more realistic especially for images with strongly varying pixel intensities. [1)]
- At a pixel dwell time of 10µs each pixel is displayed as one point in the time trace. At a pixel dwell time of 1 µs the average of 10 pixels are displayed as one point in the time trace.
- During the Test FLIM measurement the laser intensity is set that the time trace is only at rare instances hitting the red line.

Calculation of the count rate per pixel to determine the Pile-Up effect

For an already acquired FLIM measurement it is possible to determine the count rate per pixel to determine if a pile-up effect is present. Procedure:

- Calculate the exposure time per pixel: Multiply the number of frames with the pixel dwell time
- Determine the number of photons via the data reader (right mouse click) in a very bright pixel within the

FastFLIM image
- Calculate the count rate for this bright pixel: Divide the number of photons per pixel by the exposure time per pixel

Differential Count Rate

see Differential Count Rate

1)

This feature is only available in the SymPhoTime 64 Version 2.0 that is currently not available for the Leica SP8 SMD. Here, the bin width in the time trace is fixed at 1 msec averaging many pixels. This might not be suited to set the correct average count rate.

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