Correcting photon counts due to detector pile up

Background

The landmark paper for the effect on the number and brightness due to detector pile up is the paper “The photon Counting Histogram in FCS with non-ideal photo detector” by Hillesheim and Muller, Biophys J. 85, 1948-1958, 2003.

In this paper it is assumed that the PCH distribution is available. Then it is discussed how to extract the true brightness and number by performing a correction due to detector pile-up and after pulsing. Using the expressions obtained in this paper in regard to the modifications of the average and variance due to pile up, they provide the following formula for the effect on B

\[ B = 2p - 2It \]  
(equation 2)

Where B is the ratio variance to average, p is the after pulsing probability, I is the intensity in photon counts/s and t is the dead time. Ignoring for the moment the after pulsing effect, in the absence of other effects, the value of B will decrease linearly with the intensity with a slope given by 2t. The following graph is an example for the expected changes in B as a function of intensity for the avalanche photo detector (APD) with a dead time of 50 ns. We assume a sampling time of 8 us. This is typical of our mode of data acquisition.

![Effect on B due to pileup](image)

In typical conditions, B is decreased by about 0.1 at 10 counts per sampling time (8 us) for the APD. This is a very substantial amount and the data must be corrected to recover the original values. One possibility is to rotate the B vs I plot in SimFCS to account for the pile up effect.

SimFCS calculates distributions for all kind of detectors, including analog detector that have no pile up. Also the above formula is obtained at the first order, i.e., when the product of the count rate and the dead time is much less than one. In the routine for pileup correction in SimFCS we used a different approach, still based on the original assumption of the distribution being affected by the pile-up. The
reason to use this different approach is for simplicity and to maintain the inner work of SimFCS. In SimFCS, we directly correct the photon stream, rather than the distribution. We are not completely sure that the two approaches are fully equivalent. The formula we used is the standard formula for detector pile up

\[ M = C \exp(-Ct) \]  

(equation 2)

Where \( M \) is the measured counts in given dwell time, \( C \) is the theoretical counts and \( t \) is the dead time of the detector.

In all calculation we first convert the measured counts in counts/microsecond using the sampling time of data acquisition and we fixed the dead time to be 0.050 microseconds. This is adequate for the APD detector. In the future, we will provide a table for an arbitrary dead-time.

Equation 2 must be solved for \( C \), for any given \( M \) and dead time \( t \). We used a 3rd order expansion for inverting this equation.

The following graph shows the inversion and the third order interpolation used.

In the horizontal axis we have the measured counts/microseconds and in the vertical axis we have the corrected values. The formula in the figure is the formula that SimFCS uses for the correction. Of course, the counts need to be in units of counts/microseconds. The specific code follows

```pascal
function pileup(x:smallint):smallint;
var x1,x2,x3,x4,x5:double;
begin
   x1:=x*k;
   x2:=x1*x1;
   x3:=x2*x1;
   result:=trunc((0.0174*x3 - 0.0199*x2 + 1.1052*x1 - 0.0319*k)/k);
   if x1>4 then inc(hflag);
end;
```
One problem with this simple solution is that the original distribution is not continuous any more, i.e., there are values of counts that are never represented. We will evaluate the effect of this discontinuity in the future. This correction is good only for counting rates below approximately 4 MHz (for APDs). A flag will tell you if this rate has been exceeded in the data set analyzed.

**How to use the correction?**

Load a file for analysis. The correction acts on the huge vector data in both channels. In the tool menu of the ICS screen there is a new function called “correct for pile up”. Before this function can be used, you must also set the sampling frequency to whatever was used to collect the data. This is done in the field “sampling freq (pixel)” in the DISP page of the main ICS screen. For the moment, the program used the dead time for APDs set to 0.05us.
Example of data corrected for pile up (From Moreno Zamai, using the ISS ALBA, CNIC, Madrid)

Note that in this example the “intensity” varies up to 24 counts. The sampling rate was 8us/pixel, which corresponds to a peak rate of about 3 MHz. According to the formula 1, the theoretical correction due to dead time should be approximately 0.25 (up) for the point at 20 counts. This is very close to the experimental value.

Conclusions

The overall conclusion is that the correction seems to work properly and that the correction is **NEEDED** for this type of experiments.

Another conclusion is that the high sensitivity of the APD cannot be exploited for bright samples due to the very large dead time of these detectors. Already for counting rates of about 2MHz, the PMT
detector “sees more photons” than the APDs. While the APD detector is very good at very low protein expression or for solution measurements, for very bright images, it actually produces severe artifacts. These artifacts can in part be corrected with the routine in SimFCS, but the correction is only valid if the instantaneous counting rate is below 4 MHz. It seems that with the ISS Alba system this counting rate is easily exceeded.

50 ns, sampled at 400KHz $y = 1.162E-04x^5 - 4.366E-03x^4 + 5.969E-02x^3 - 3.022E-01x^2 + 1.572E+00x$

50 ns, sampled at 40KHz $y = 1.162E-08x^5 - 4.366E-06x^4 + 5.969E-04x^3 - 3.022E-02x^2 + 1.572E+00x$

50 ns, sampled at 1000000 $y = 4.539E-03x^5 - 6.822E-02x^4 + 3.731E-01x^3 - 7.555E-01x^2 + 1.572E+00x$

50 ns, sampled at 100000 $y = 4.539E-07x^5 - 6.822E-05x^4 + 3.731E-03x^3 - 7.555E-02x^2 + 1.572E+00x$