

Supplementary Information

The link for the LabVIEW program:

<https://github.com/SadatHasan/EasyFlux.git>

Calculation of average lifetime:

The average lifetime is calculated from the average number of photons in the histogram bin. The equations are taken from recent publications.^{1,2} The histogram bin with the average number of photons is given by:

$$\bar{v} = \frac{\sum_i^m i \cdot N_i}{\sum_i^m N_i}$$

Here i refers to the histogram bin index, m is the total number of bins and N is the number of photons in i -th bin. Once the index has been obtained by the sub-

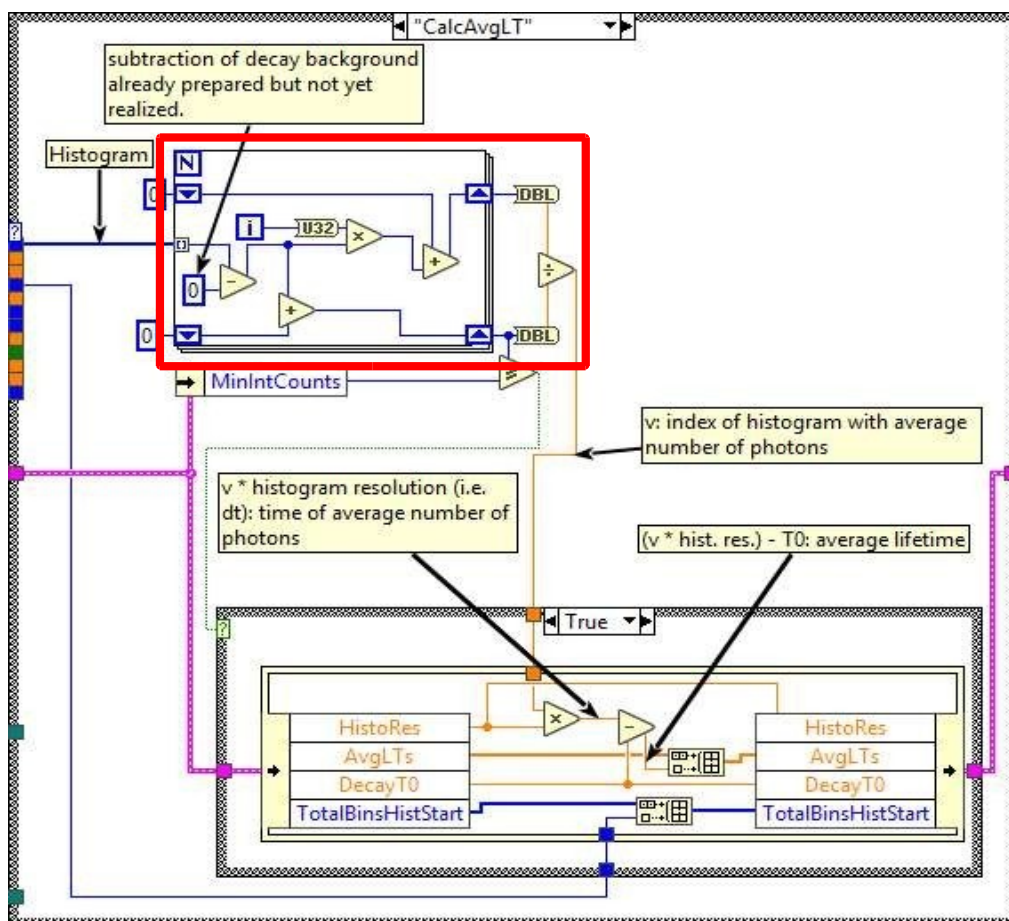


Figure 1 Sub VI for calculating average lifetime

VI, multiplying it with the histogram resolution (i.e. value you set by 'Resol. ps' in 'Inp. Chan 1' box on EasyFlux front panel) provides the average photon time.

This equation is realized in case "CalcAvgLT" of EF_AnalysisThread.vi (red box):

The program has been designed to get the T0 value from the user. As a result, the decay is not initiated at $t = 0$ but at $t = \text{DecayT0}$ (i.e. value set by user 'DecayT0 ns' in the histogram box on EasyFlux front panel or by dragging the green cursor on the histogram display), this value needs to be subtracted from the average photon time to give the average lifetime of the histogram (i.e. the fluorescence decay time in case of a mono-exponential decay). The user is responsible for setting a threshold for this average lifetime that eventually functions as the sorting criteria and generates the TTL pulse.

Remark 1:

'DecayT0' is currently set by the user. If the laser IRF is drifting over time (i.e. the onset of the decay curve shifts over time), this value needs to be adjusted. Alternatively, it can be calculated for every decay (e.g. by setting DecayT0 to histogram time, where number of photons is e.g. 10 % (or other value) of maximum number of). This would potentially eliminate the influence of IRF shifts but introduce some additional noise to the calculated average lifetimes.

Remark 2:

At the moment the background of the decay is not taken into account for calculating the average lifetimes (the sub-VI is available in the program but non executing). This can introduce a systematic error for larger background values.

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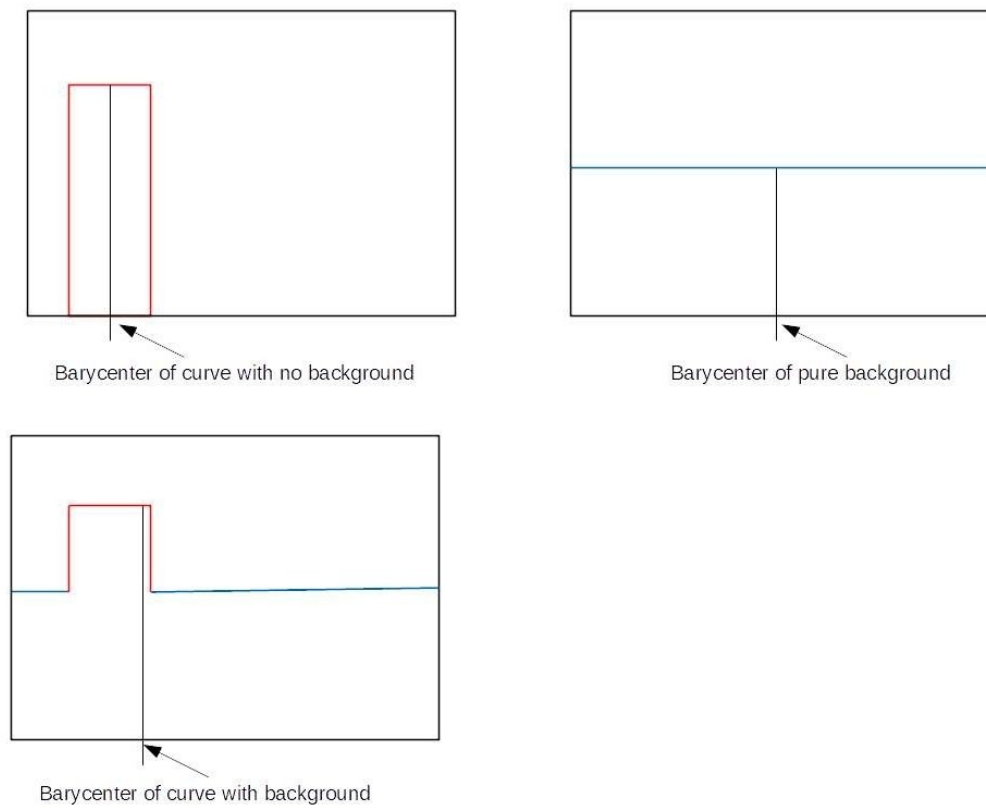


Figure 2 Generalized sketch to demonstrate how the background shifts the average lifetime towards values which are situated in the middle of the histogram. The larger the background, the stronger is this effect

Reference:

- 1 T. Lieske, W. Uhring and N. Dumas, *Des. Archit. Signal Image Process.*, 2017, 1–6.
- 2 E. Fišerová and M. Kubala, *J. Lumin.*, 2012, **132**, 2059–2064.