

## **NovaFLIM Calculated IRF**

In the Nova Flim the IRF is parameterized as a combination of two Gaussian curves (a total of 5 fit parameters). These parameters are adjusted during Initial Fit.

Roughly speaking, the IRF (Instrument Response Function) can be approximated as a sum of two Gaussian curves.

The first Gaussian curve is actually a composite assymetric curve, as it can have two different standard deviations (sigma). Three of the five parameters describe this first Gaussian curve: the position of its maximum time-position on the x-axis, sigma1, and sigma2.

- X-position of the maximum of Gaussian curve 1
- Sigma1 (standard deviation for the rising edge) of Gaussian curve 1
- Sigma2 (standard deviation for the falling edge) of Gaussian curve 1

To explain further: up to the maximum point, the curve behaves like a Gaussian with sigma1; beyond the maximum, it follows a Gaussian with sigma2. This allows for different rise and fall characteristics, reflecting the behavior observed in reality.

A second Gaussian curve is added, with its standard deviation defined as the average of sigma1 and sigma2; i.e., (sima1+sigma2)/2. The fitting parameters are:

- X-position of the maximum of Gaussian curve 2
- Height of Gaussian curve 2 (limited to the height of Gaussian curve 1)

The IRF is determined only during the initial TCSPC fit, which means it may vary depending on the different exponential components. During the pixelwise fitting in FLIM, this IRF is applied without any modifications. Consequently, all image points are fitted using the same fixed IRF established during the initial fit.

This model is the result of extensive internal development, testing, and refinement. By optimizing these parameters, the IRF model can accurately represent the measured response of the system.

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