OPTIMISATION OF FRET MEASUREMENT IN PHASOR BY IMAGE SEGMENTATION

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Fluorescence lifetime imaging microscopy (FLIM) is a key technique to observe and quantify the behaviour of a fluorophore in different conditions. This technique has taken a main role in the observation of interaction between two proteins by reporting the Forster resonance energy transfer (FRET).

The standard fitting method has been largely used for estimating the fluorescence lifetime. However, obtaining reliable results with this fitting approach is time consuming and requires a high level of expertise. Recently, many efforts have been done to simplify the analysis of FLIM images and to make it accessible to the non-expert user or for High Content Microscopy approach. Among all these techniques the polar plot or phasor is increasingly used. We have adapted this polar approach in dedicated software for FLIM Analysis (MAPI) [1]. We have previously demonstrated that the Polar approach enable to determine more precisely the lifetime values for a limited range corresponding to usually encountered fluorescence lifetime values.

However the dynamic range in living cells depend on the heterogeneity of the fluorescence and the signal/noise ratio per pixel, for example when targeting in cellular compartments. In order to optimise and increase the robustness of FRET measurements, we integrate three tools of segmentation by number of photon in each pixel:
- Maximal channel photon number
- Integral photon number
- Acceptor intensity

We compare the reproducibility and variability of lifetime measurements obtained with these techniques for simulated and real data of FLIM. We discuss about the advantages of each techniques for biological applications. Finally by the mean of these techniques, we study proteins interactions in different cell compartments such as mitochondria, endoplasmic reticulum and nucleus.


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