INVESTIGATION OF THE POLAR REPRESENTATION FOR TD-FLIM

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KEY WORDS: Fluorescence lifetime imaging microscopy (FLIM), Forster resonance energy transfer (FRET), phasor, time-correlated single-photon counting (TCSPC).

Fluorescence Lifetime Imaging Microscopy (FLIM) is a well established technique which is routinely performed in many laboratories. Since the fluorescence lifetime is sensitive to the local environment of the fluorophore (e.g. [Ca$^{2+}$], pH, temperature,…), FLIM was largely used to explore dynamic interactions between proteins by detecting lifetime modifications associated with Förster Resonance Energy Transfer (FRET) occurring between two fluorescent probes.

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. Among all these techniques, the polar plot or phasor is increasingly used [1-2].

We have investigated the performance of this polar approach and compare it with the standard fitting method. Interestingly, we have demonstrated theoretically that the standard fitting method does not provide the best estimator of the lifetime for fluorophores exhibiting mono-exponential intensity decays as soon as some fluorescence background is present [3]. The polar approach enables indeed to determine more precisely the lifetime values for a limited range corresponding to usually encountered fluorescence lifetime values.

We have also investigated the influence of several experimental parameters (i.e. instrumental response function, background, angular frequency, number of temporal channels) on the polar calculations and suggest parameter optimization for minimizing errors. We have for instance demonstrated that the numerical integration method employed for integrals calculations may induce errors when the number of temporal channels is low. We report theoretical generalized expressions to compensate for these deviations [4]. These theoretical generalized expressions were finally corroborated with both Monte Carlo simulations and experiments.