# MULTISPECTRAL FLIM ACQUISITION AND 3D POLAR REPRESENTATION

**A. Leray, C. Spriet, D. Trinel, L. Héliot**

**Interdisciplinary Research Institute CNRS USR 3078**
**Science and Technology University of Lille**
**Parc de la Haute Borne 59650 Villeneuve d'Ascq (FRANCE)**
**E-mail : aymeric.leray@iri.univ-lille1.fr**

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Fluorescence Lifetime Imaging Microscopy (FLIM) is a well established technique to differentiate molecular species spectrally undistinguishable and to map the local modification of the label environment (ions concentration, pH…). FLIM was also largely used to explore conformation change of proteins or to separate interacting and non interacting molecular fractions in Förster Resonance Energy Transfer (FRET) experiments. However due to the optical properties of the biological tissue (which are absorbing, highly scattered, heterogeneous and autofluorescent), it is necessary to use an up to date system coupling spectral and lifetime measurements and named multispectral FLIM [1].

The fast analysis of the multispectral FLIM images constitutes a major challenge which required global analysis and optimized algorithm. Due to the number of mathematical algorithm and due to the correlation between lifetimes and contribution, obtaining reliable results with this fitting approach is time consuming and requires a high level of expertise. Recently, many efforts have been done to help the user to simplify the conclusion in classical FLIM experiments [2-4].

We present here for the first time, an approach that considerably simplify the analysis of multispectral-FLIM images avoiding complex fitting algorithm strategies, and permitting a fast and visual graphical representation of the fluorescence lifetimes. By transforming the experimental data from time domain to frequency domain for each spectral channel, we calculate the 3D polar representation. We apply it in particular on FRET experiments and demonstrate that quantitative FRET measurements with a high level of precision can be performed [5]. Analysis artifacts occasioned by fitting algorithm choice become then totally inexistent.

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