

## Time-Correlated Single Photon Counting-based FLIM, FRAP and FAIM to investigate intracellular dynamics and their environments

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In fluorescence microscopy, obtaining as much information as possible in a single experiment is of great interest. This approach decreases possible damage of the biological sample and increases the information obtained from the limited photon budget before the fluorophore is irreversibly bleached. Here, we report on using different Time-Correlated Single Photon Counting (TCSPC) techniques simultaneously under the same conditions [1: Time-Resolved Fluorescence Lifetime Imaging (FLIM), Time-Resolved Fluorescence Anisotropy Imaging (tr-FAIM), and Fluorescence Recovery After Photobleaching (FRAP). FLIM yields information about the fluorescence lifetime, which can be sensitive to the microenvironment of the fluorophore. Tr-FAIM can monitor the depolarization of the fluorescence, which can be due to rotational motion, or homo-FRET. FRAP shows the translation of the fluorophore [2]. We have used this approach to study GFP-monomer and GFP-dimer constructs in different PBS-glycerol solutions. The characterization of these two models demonstrate the potential of this approach, and will serve us as a reference for the interpretation of homo-FRET data of protein dimerization within cell membranes.

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