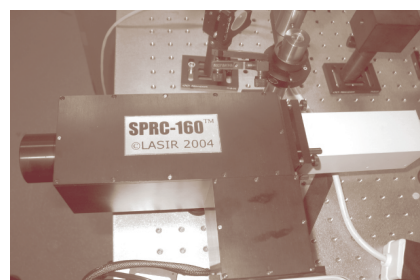


## Spectrally resolved fluorescence lifetime measurements to determine protein-protein interactions in living cells.

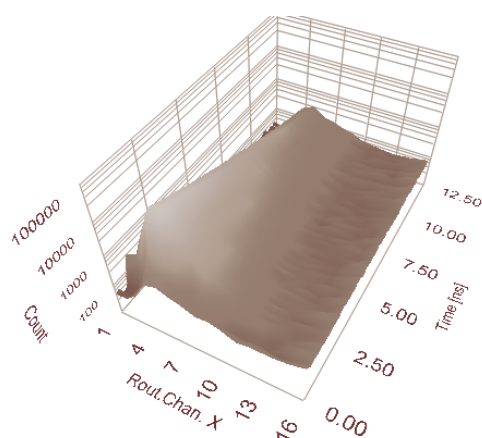
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Studying protein-complexes dynamic formation and dissociation is an important step towards the functional understanding of living cell. FRET measurement allows us to perform those studies by analysing variations in parameters like fluorescence lifetime [1] or spectral [2] properties of tagged proteins. However, it is often difficult to do precise and quantitative analysis of the obtained results, giving us only a qualitative response. To go further, we decided to correlate results from both of those techniques, and so, to build a system allowing us to collect those informations: the SPRC-160. In this work, we present



**Figure 1: picture of our home-made lifetime and spectral measurements system the SPRC-160**



**Figure 2: example of smoothed data from a CFP-YFP linked protein.**

the set-up and characterisation process to perform simultaneous and localized fluorescence lifetime and spectral acquisitions in living cells. This system is based on the Leica SP2 confocal microscope coupled to a Mira 900M femtosecond pulsed laser allowing two photon excitation, and so, minimizing invasivity due to fluorophores excitation [3]. We used the descanned port of this microscope to adapt our homemade system (figure1), using optimized spectral dispersion of fluorescence emitted light on a 16 channel photomultiplier. The routing and time channels from the detector and the synchronisation signal from the laser are fed in a TCSPC card (SPC730, Becker and Hickl) giving us a 2D matrix of photon emission time in x and wavelength values in y (figure2).

Fluorescence lifetime and spectra are then extracted and analysed from those matrices and will be correlated to investigate FRET occurrences in our samples.

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[3] Squirrell JM, Wokosin DL, White JG, Bavister BD, “Long-term two-photon fluorescence imaging of mammalian embryos without compromising viability.” *Nat Biotechnol*, 17(8):763-7. (1999)