Quantitative FRET to study mechanotransduction

Alexis Coullomb, Cécile Bidan, Aurélie Dupont – LIPHy, Grenoble

Fluorescence Resonance Energy Transfer (FRET) imaging techniques allow us to measure in real time and in live cells distances between two fluorophores. Thanks to ingenious constructions implying spring-like proteins or kinase substrates, FRET efficiency can be used to monitor molecular forces or the activity of a signaling proteins respectively. Unfortunately, most measurements are only qualitative, and it is hard to compare results between cells, experiments and laboratories.

Here we propose a method using alternating laser excitation (ALEX FRET) to quantitatively measure the FRET efficiency and fluorophores stoichiometry. We correct for the cross-talk of donor fluorophores into the acceptor channel, and for the direct excitation of acceptor fluorophores during the excitation of donors. Two other factors remain to be determined: the gamma factor, that corrects the differences in fluorophore's quantum yields and photon detection efficiencies, and the beta factor, that accounts for the differences in excitation intensity and absorption cross-section of the fluorophores. These two factors are more challenging to estimate, and we will discuss the different ways to evaluate them.