Biological processes are intrinsically dynamic. Although traditional methods provide valuable insights for the understanding of many biological phenomenons, the possibility nowadays to measure, quantify and localize proteins within a living cell has revolutionized our train of thoughts and has encouraged scientists to develop molecular tools for the assessment of protein or protein complex dynamics within their physiological context. We focus on genetically-encoded biosensors and specifically those dedicated to kinase activity measurements [1] of the MAPK/Erk pathway in a specific form of programmed cell death, the necroptosis [2]. However, depending on cell type and stimulus, compartmentalized MAPK activity will also mediate either cell survival or cell death. Thus, spatial and temporal signatures for the signal propagation of the MAPK/Erk signaling pathway have started to be explored to understand how a cell engages survival responses rather than cell death. To quantify kinase activity in the particular case of biosensor application, the fluorescence lifetime imaging (FLIM) is a very interesting alternative and was used in our study. FRET FLIM experiments measure the donor fluorescent protein lifetime changes which make it extremely valuable to measure activity of the kinase MAPK/Erk in this cellular context.
