Fluorescence Lifetime Imaging (FLIM) in combination with a variety of fluorescence techniques is becoming an important tool in cell biology for the study of dynamic interactions of molecular species, for example protein-protein interactions, and for the intracellular mapping of ion concentration and pH imaging. We present FLIM data obtained with a new method called “Digital Frequency Domain” (DFD). It offers superior sensitivity, which results in a factor of four reduction in the time required for image acquisition as compared to the traditional approach using time-correlated single photon counting (TCSPC). This improves capturing images of motile samples, e.g., live cells.

We report the measurement of Förster Resonance Energy Transfer (FRET) efficiency by using FLIM on mouse pituitary GHFT1 cells labeled with Cerulean (donor) and Venus (acceptor) fluorescent proteins. Several genetic constructs were developed tethering these fluorescent proteins via different amino acid linkers. A standard ISS FastFLIM unit was attached to an ISS Alba confocal FLIM microscope using one-photon excitation. The energy transfer efficiency was measured as a function of the FRET pair separation distance. Data analysis was achieved by using the phasor plot approach. Values for the FRET efficiencies could rapidly be obtained, which allowed for an immediate visualization of the location of FRET processes.