The photophysical phenomenon of Förster Resonance Energy Transfer (FRET), i.e., the radiationless transfer of excited state energy between two spectrally matched fluorophores, provides an extremely powerful method for the detection and quantification of protein-protein interactions and protein conformational changes in biological specimens. This tutorial will focus on the instrumentation and analytical needs to perform FRET microscopy, and showcase how molecular-scale information, derived by FRET, can be used to construct optical bioassays for the investigation of cellular biochemistry and physiology. Guidelines for experimentation will be provided, also on the basis of practical examples from biological applications.

An important optical technique in the quantitative use of FRET measurements in the microscope is based on the interrogation of the excited state decay behavior of fluorophores using Fluorescence Lifetime Imaging (FLIM). This technique is becoming increasingly available to imaging labs and core facilities in the life sciences as routine add-on to existing microscope setups and comes with unique advantages. The tutorial will cover these aspects, its different implementations, and will offer a perspective for technical developments and possibilities in the near future.