

## TUTORIAL WORKSHOP: FLIM AND FRET TECHNIQUES.

**T.W.J. Gadella Jr.**

**Section of Molecular Cytology & Centre for Advanced Microscopy,  
Swammerdam Institute for Life Sciences, University of Amsterdam,  
Kruislaan 316, NL-1098 SM Amsterdam, The Netherlands  
Email: Th.W.J.Gadella@uva.nl**

The application of Förster Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging Microscopy techniques (FLIM) have expanded exponentially over the past years; these techniques become an increasing staple in many biological and biophysical fields of application. The reason for this enormous uplift are i) the ease of in situ fluorescence labeling using the visible fluorescent proteins; ii) the commercial availability of advanced fluorescence microscopes with FRET acquisition software capable of acquiring complete spectra or fluorescence lifetimes; and most importantly iii) the unique information on in situ molecular conformation and –proximity that FLIM and FRET can extract from single living cells. However, for non-FRET experts, the underlying principles and pitfalls are often not well understood.

This tutorial is especially of interest for those scientists applying FRET and FLIM techniques for biological research but who want to know more about FRET and FLIM basics and the type of applications that are currently possible. Several aspects on theory, instrumentation and application will be part of the tutorial with reference to the recently published book on FRET and FLIM techniques [1].

### **Lecture 1: FRET basics [1].**

Spectroscopical definition of FRET, distance dependence, definition of Förster radius, experimental determination of overlap integral, theoretical modes of FRET detection by donor Fluorescence intensity and lifetime, sensitized acceptor fluorescence, donor- and acceptor photobleaching, FRET probes.

### **Lecture 2: FRET-(FLIM) microscopes [1]**

Description of time-domain and frequency-domain techniques and their implementation into the fluorescence microscope, quantitative filter FRET and spectral unmixing FRET systems.

-Break

### **Lecture 3: (Advanced) biological applications: seeing is believing.**

Description of our own recent work on FRET and FLIM including FRET competition for multimeric systems,  $\kappa^2$ -FRET-FLIM, TIRF-FLIM-FRET [1], and dual & triple lifetime unmixing-FLIM using polar plots.

[1] T.W.J. Gadella jr (ed) *FRET and FLIM techniques* 534 pp, in *Laboratory Techniques in Biochemistry and Molecular Biology* vol 33 (P.C. van de Vliet & S. Pillai, series editors), (Elsevier, Oxford, 2009).