New possibilities for interactive specification and validation of models for Fluorescence Lifetime Imaging Microscopy (FLIM) data with the TIMP package.

Laptenok P. Sergey\textsuperscript{1,3}, Katharine M. Mullen\textsuperscript{2}, Jan Willem Borst\textsuperscript{1}, Herbert van Amerongen\textsuperscript{1,3}, Antonie J. Visser\textsuperscript{1}

\textsuperscript{1} MicroSpectroscopy Centre, Wageningen University and Research Center, The Netherlands
\textsuperscript{2} Department of Physics and Astronomy, Faculty of Sciences Vrije Universiteit Amsterdam, The Netherlands
\textsuperscript{3} Laboratory of Biophysics, Wageningen University and Research Center, The Netherlands

The detection of protein-protein interactions in a biological cell is required to enhance our knowledge about mechanisms that regulate intracellular processes. Förster Resonance Energy Transfer (FRET) between donor and acceptor molecules is a widely used technique to monitor protein-protein interactions. As FRET is a fluorescence quenching process, it can be detected by the shortening of the fluorescence lifetime of the donor molecule. Fluorescence Lifetime Imaging Microscopy (FLIM) allows the mapping of fluorescence lifetimes with (sub-){\textsuperscript{}} nanosecond time resolution and a spatial resolution of 250 nm. FRET phenomena measured with the FLIM technique provides temporal and spatial information about molecular interaction in living cells.

For accurate and quantitative FLIM data analysis well-designed analysis protocols are required. The dynamical features of a FRET system are often well described by a small number of kinetic processes, in which the associated fluorescence lifetimes in all pixels have similar values, but the relative amplitudes may vary from pixel to pixel. In this case significant advantages and accuracy in analysis can be achieved by global analysis of the image. Global analysis uses fluorescence decay traces from all pixels to estimate both kinetic parameters (lifetimes) and relative amplitudes of components in each pixel. The TIMP package has been shown to be effective at performing global analysis of FLIM images \cite{1}.

A typical FLIM image represents in the order of $10^3$ pixels with $10^3$ time points per pixel. Presentation of the data analysis results needs to be well-organized and interactive, allowing the user to obtain a detailed graphical presentation of the fit at any pixel selected. Here we present new options for the TIMP package allowing interactive presentation of global analysis results, as well as importing and preprocessing FLIM data. The analysis of FLIM images of transcription factors fused with either cyan fluorescence protein (CFP) or yellow fluorescence protein (YFP) in plant cells will be given as an example. The novel data analysis methodology could reveal molecular interactions among different transcription factors in the nucleus of a plant cell.

References: