

Tips & Tricks : FLIM Microscopy

Conventional microscopy uses fluorescence intensity to obtain information on the location and relative concentration of targets in a sample. These information can be improved by fluorescence lifetime microscopy (FLIM). FLIM can be used to detect environmental changes, protein interactions and spectral separations. This technology is available at the GIGA Cell Imaging platform, thanks to the Leica Stellaris 8 and the recent acquisition of the FALCON module, which makes FLIM technology even more accessible and easy to use.

How the FLIM microscopy works

FLIM microscopy requires two important elements in a microscope linked to the measurement of the fluorophore's lifetime between its excitation (pulsed laser) and the emission of its photon (detector with a short dead time). For a good acquisition, you need to accumulate a large number of photons per pixel, and therefore perform many iterations of the same image to obtain a sufficient number of photons to analyze the FLIM data. In the case of our Stellaris 8 FALCON, two things make FLIM more accessible : the pulsed white light laser, which make excitation at the maximum of intensity for various fluorophores and therefore creates a higher photon emission, combined with the power contingent of Leica's Hybrid detectors, drastically reduces the number of iterations required.

Power Counting system

Conventional counting: 2 photon events separated in time, 2nd photon arrival below the pulse pair resolution, 1 count missing.

Power Counting: 2 overlapping photon events enabled by pulse width measurement, Recognition of 2 overlapping photon events enabled by pulse width measurement.

Moreover, the FALCON module makes it easy to acquire and analyze FLIM data, thanks to the phasor analysis system.

FRET with FLIM Microscopy

The most widely used application for FLIM is FRET (Fluorescence Resonance Energy Transfer). FRET is often used to observe protein-protein interactions. This technique involves exciting a donor fluorophore, which then transmits its emission photon to excite the acceptor fluorophore. This is possible if the fluorophores are at a distance of less than 10 nm, characteristic of a protein-protein interaction. In the case of a FRET measurement without FLIM, we'll calculate a ratio of

$$R_{fret} = \frac{Intensity_{emAcceptor}}{Intensity_{emDonor}}$$

which is sensitive to photobleaching and therefore results in an imperfect, indirect FRET measurement. With FLIM, only the donor lifetime is observed, which will decrease in the event of protein-protein interaction and hence FRET. The advantage of FLIM lies in its more direct measurement, which is not dependent on the photobleaching of fluorophores. The decrease in donor lifetime during FRET action can be explained by the fact that the donor fluorophore has a different pathway to move from its excited state S1 to its stable state S0. This new pathway increases the number of photons that return to a stable state at a given time t, and therefore reduces the fluorophore's lifetime.

Describing your environment

FLIM microscopy can be used to observe various types of environmental changes.

Combined with fluorophores sensitive to environmental changes such as pH, calcium flux, ion concentrations or NADH NADPH.

They can be observed by varying the lifetime of the bound fluorophores or by fluorescence.

Observation of pH variation with FLIM microscopy

Lifetime can be used to separate autofluorescence or reflection of a tissue or material from your fluorescence signal.

Separation of autofluorescence from fluorescent signal using FLIM microscopy

Going further with FLIM microscopy

Fluorescence lifetime detection and its variations can be used in a variety of different ways. One of these ways is multiplexing. Each fluorophore has a different lifetime, so it's possible to accumulate several fluorophores in the same spectral range. This can be useful for reducing phototoxicity during time-lapse.

Separation of spectrally identical signals according to their lifetimes

Some fluorophores have been created to observe specific phenomena in cells, such as Flipper TR, which measures cell membrane tension as a function of lifetime.