New Insight in Cellular Signaling from Fluorescence Lifetime

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Functional imaging plays a major role in giving mechanistic insights at the molecular level driving our understanding of cellular processes. Fluorescence microscopy and fluorescence lifetime imaging (FLIM) are methods of choice because of their sensitivity and multiplexing possibilities.

By exploiting the spectral dimension and FLIM it is possible to "see more". Lifetime gives access to biosensing (e.g. ion concentrations, metabolic states) since it depends on the fluorophore micro-environment, to molecular interaction through FLIM-FRET (1), and to multiplexing through lifetime-based species separation (e.g. determination of fluorescence signal vs. autofluorescence).

In this talk we will present several examples on how lifetime imaging enables more insights into biological processes. We will show how a novel mutation in a Zn2+ transporter (ZIP7) is responsible for a newly described immunodeficiency syndrome (1). This work on primary mice pre-B cell is based on ZIP7 mutations recreated via CRISPR-Cas 9. The FLIM data from the eCALWY-4 and eCALWY-6 biosensors (2, 3) provided the cytosolic vs. ER localized Zn2+ dynamics. These imaging experiments allowed to see how ZIP7 is responsible for maintaining the Zn2+ gradient between the ER and the cytoplasm that is key for B cell development.

We will show how the novel FLIM approach in FALCON (FAst Lifetime CONtrast) with its speed and integration has been key to generate mechanistic information at confocal speeds, compatible with fast cellular processes (4).

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