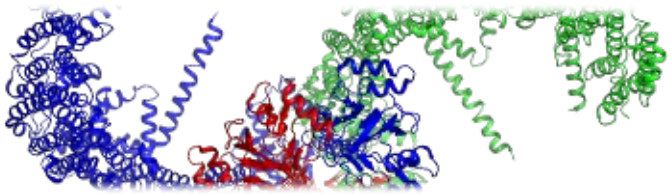

BPS2023
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SAN DIEGO
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Monday, February 20
12:30 PM – 2:00 PM
Room 10
Leica Microsystems

Molecular interaction across imaging modalities with the Leica STELLARIS confocal platform
Molecular interactions and dynamics are the foundation of cellular machinery. For molecules to interact, they must come close enough in space so they can exert a chemical influence on their molecular environment. Confocal fluorescence imaging became a pillar in such endeavors for the specificity conferred by fluorochromes, and the spatial context, spatial and temporal resolution for the inherent dynamic nature of the interaction process.

The ultimate tool of choice to study the interaction of two molecules is Förster Resonant Energy Transfer (FRET), where the molecules of interest are tagged with probes that act as a "donor-acceptor pair" (1).

Many FRET approaches rely on intensity-based measurements such as quenching of donor, acceptor photobleaching, and sensitized emission. Intensity-based FRET requires basic instrumentation but has high risks of artifacts from experimental design.

The gold standard to measure FRET is FLIM (fluorescence lifetime imaging microscopy), as the FRET readout here depends only on the donor fluorescence lifetime changes. The analysis of FLIM-FRET can be done by fitting the fluorescence decay with a multi-exponential model or non-fitting methods, such as phasor FLIM (2) and minimal fraction of donor molecules (mf_D) approach (3). All these approaches are available in the STELLARIS confocal platform. In particular, mf_D has been implemented as a TauSense tool named TauInteraction.

The advanced tools of STELLARIS confocal platform are also available under cryogenic conditions, enabling advanced cryo correlative (cryo-CLEM) studies. Here, new cellular mechanisms can be unraveled. Intracellular protein interactions can be further resolved by cryo electron microscopy (EM) down to the subnanometer scale. To increase the success rate of these types of studies, Cryo light microscopy enables the identification of specific target proteins prior to the EM imaging. LIGHTNING and TauSense provide high accuracy in identifying and targeting the desired proteins for further analysis.

Speaker
Giulia Ossato, Senior Product Manager, Leica Microsystems