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INM COLLOQUIUM

"SPECTRAL IMAGING, FLIM-FRET, FRAP AND STED:
SEEING IS UNDERSTANDING"

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Monday, February 21, 2022, 09:00 p.m.

Remote via Zoom: https://us06web.zoom.us/j/89228064417

Host: Prof. Dr. Aránzazu del Campo

Fluorescence microscopy is a powerful tool for modern multidisciplinary scientific research. A wide range of microscopy techniques are developed for unraveling scientific questions. Four imaging techniques and their applications will be discussed during this meeting. First, spectral imaging combined with linear unmixing allows the reliable separation of strongly overlapping fluorophores into pure signals. This technique was utilized for multicolor imaging as well as elimination of autofluorescence in tissues. Second, fluorescence Lifetime Imaging (FLIM) in combination with Förster Resonance Energy Transfer (FRET) has been proven to be an effective method for studying molecular interactions. FLIM-FRET and tension sensor were used to visualize mechanical forces across VE-cadherin-catenin complex at endothelial cell-cell junctions. Third, Fluorescence Recovery After Photobleaching (FRAP) is a widely used tool for measuring diffusion in biological samples. Particularly, FRAP was optimized to study the mobility of VE-cadherin-EGFP in HUVECs under various conditions. Finally, Stimulated Emission Depletion Microscopy (STED) allows for the visualization of fluorescence structure with a spatial resolution below the diffraction limit. STED creates sub-diffraction limit structures by selective deactivation of fluorophores, reducing the area of illumination at the focal point. As the standard confocal microscopy was insufficient to resolve the nanoscale distribution of two molecules (β-catenin and plakoglobin) at cell-cell junctions, STED was used to visualize the localization of these molecules in great detail.

KONTAKT

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