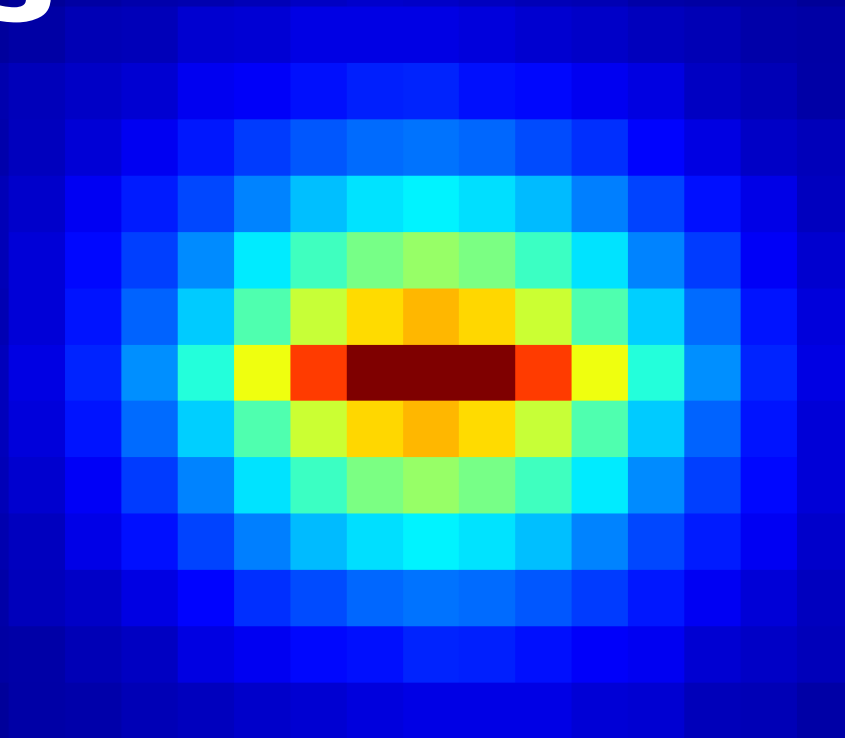


Uncover the true behavior of proteins in living cells



ZEISS Spectral RICS

Your Solution for Mapping Molecular Interactions in the Cellular Environment

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Seeing beyond

ZEISS Spectral RICS

Uncover the True Behavior of Proteins in Living Cells

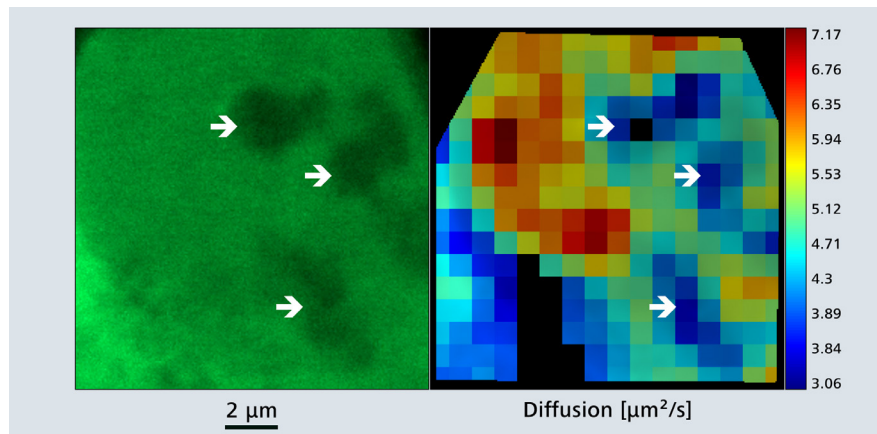
While fluorescent imaging of living cells offers a multitude of information about sub-cellular structures and organelles, understanding protein-level behavior can be challenging. ZEISS Spectral RICS combines LSM imaging with information about the behavior of proteins in their cellular environment. In addition to obtaining structural image information of your sample, you can also unveil how proteins of interest move and interact in relation to their position within the cell. This integrated approach facilitates the identification of regions exhibiting diverse molecular characteristics. Uniquely, through spectral unmixing, Spectral RICS provides an optimal foundation for investigating protein-protein binding behavior.

Raster Imaging Correlation Spectroscopy (RICS) is an advanced technique used in biological imaging to study the dynamics of molecules and particles at the cellular level. It involves analyzing the movement of fluorescently labeled particles across a raster-scanned image of a sample. By correlating the intensity fluctuations between different pixels in the image, RICS can provide information about the diffusion rates, concentration, and interactions of these particles.

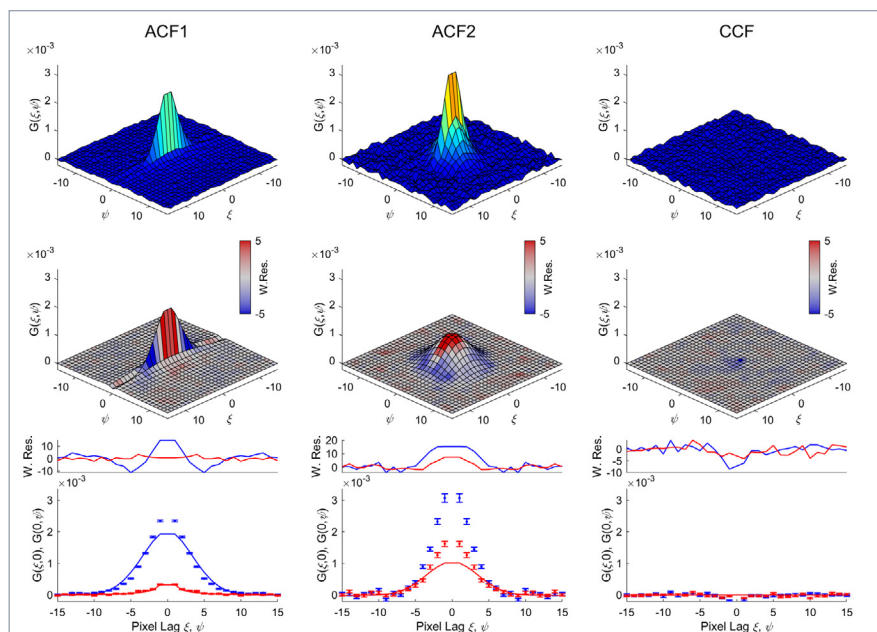
The advantage of Spectral RICS

ZEISS Spectral RICS has been developed in collaboration with Prof. Jelle Hendrix, Hasselt University, Belgium. It adds spectral unmixing to the RICS method, helping you to avoid the misinterpretation of overlapping spectra as protein interactions. With unmixed spectra, you can be sure to obtain unbiased information and reveal the true behavior of proteins in living cells.

ZEISS Spectral RICS comes as a workflow-based ZEN extension, guiding you from experiment setup to data analysis. It includes spectral unmixing, auto and cross correlation analysis, intensity and grid heatmaps, and time-dependent analysis, combined with tools such as ROI selection and intensity thresholding.



RICS example: Raw time series (left) and grid heatmap (right). The RICS analysis reveals that EGFP diffusion is slower inside the nucleoli compared to the nucleoplasm. Sample kindly provided by P. Hemmerich and T. Ulbricht, Core Facility Imaging, Leibniz Institute on Aging, Jena, Germany.



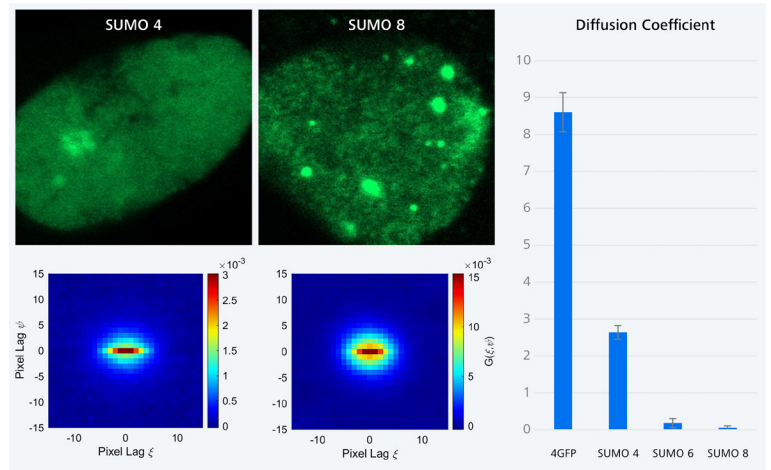
Spectral RICS example: An assumed interaction between two proteins could be proven to be non-existent with RICS analysis after spectral unmixing. Sample kindly provided by Prof. Jelle Hendrix, Dynamic Bioimaging Lab, Advanced Optical Microscopy Centre, Biomedical Research Institute, Hasselt University.

ZEISS Spectral RICS

Application Examples

Effects of SUMOylation in protein diffusion

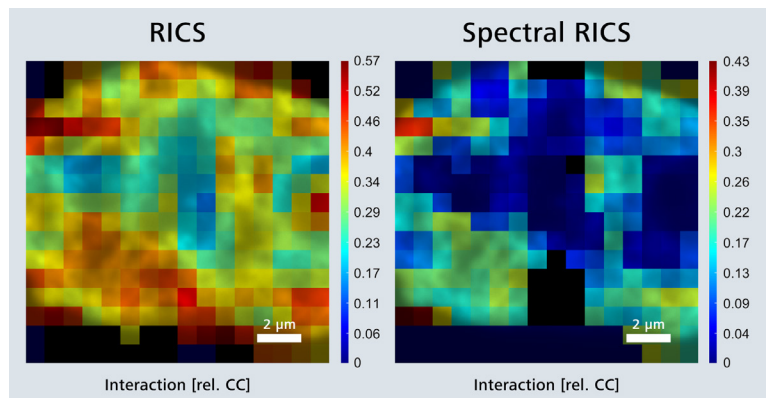
RICS can be used to measure changes in diffusion resulting from protein interaction. With standard auto-correlation RICS analysis, we can see that the diffusion coefficient drops in correspondence with the size of SUMO chain. This type of studies can also measure the changes in diffusion of tagged proteins of interest in the presence of drug treatments, mutations, or other influences.



Samples kindly provided by P. Hemmerich and T. Ulbricht, Core Facility Imaging, Leibniz Institute on Aging, Jena, Germany

Interaction of LEDGF with histones

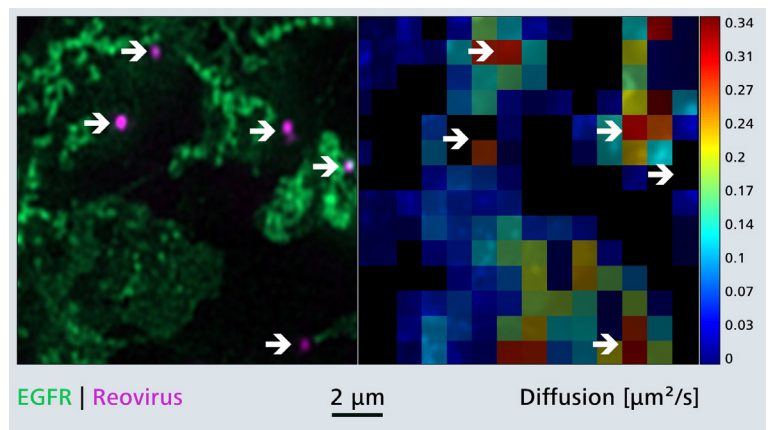
LEDGF is a chromatin binding transcription factor, and H2B is a histone that helps organize eukaryotic DNA. Before spectral unmixing, RICS cross-correlation analysis of the 2 proteins shows strong interaction. However, spectral unmixing reveals that in most of the nucleus the interaction of the two proteins is very small.



Sample kindly provided by Prof. Jelle Hendrix, Dynamic Bioimaging Lab, Advanced Optical Microscopy Centre, Biomedical Research Institute, Hasselt University

Exploring the behavior of EGFR at reoviral docking sites

Epidermal Growth Factor Receptor (EGFR) is expressed on the membrane of cells and is one of the entry receptors for reoviruses. The reoviral landing and docking sites are visualized in magenta. RICS analysis depicted as a grid-based heatmap reveals that the diffusion of EGFR is faster adjacent to the viral docking sites, compared to other membrane locations.



Sample kindly provided by J.D. Simpson & D. Alsteens, NanoBiophysics lab, UC Louvain, Belgium

ZEISS Spectral RICS

Integrate Confocal Imaging with Molecular Characteristics

ZEISS Spectral RICS features

- ✓ CPM Measurement to support optimal hardware adjustment
- ✓ Calibration of confocal volume to determine absolute molecule concentration
- ✓ Spectral Unmixing to clearly separate emission signals for cross-correlation analysis
- ✓ Selection tools like ROI definition and thresholding to focus data analysis
- ✓ Visualization of fit results in 3D plots
- ✓ Grid or intensity based heatmaps to easily visualize concentration or diffusion differences in the imaged area
- ✓ Time dependent analysis to register temporal changes throughout the measurement
- ✓ Results table including parameters which indicate quality of input data and fit result

Compatibility

ZEISS LSM 980	ZEN 3.9 system software or ZEN Desk* 2D Toolkit
Objective lenses	C-Apochromat 40x / 1.2 W Corr FCS C-Apochromat 63x / 1.2 W Corr FCS

* with ZEN Desk Spectral Unmixing and Data Analysis possible

Related Products:

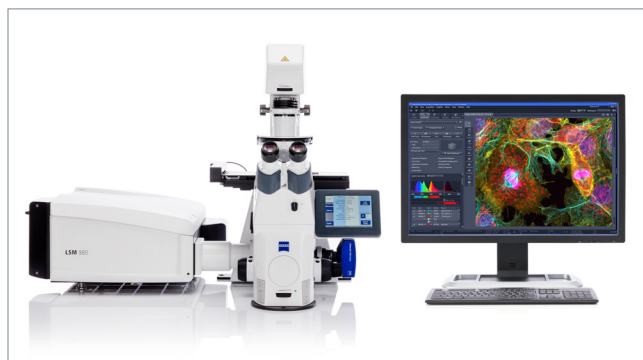


ZEISS Dynamics Profiler

Your Easy Access to Underlying Molecular Dynamics in Living Samples

Uncover molecular concentration, asymmetric diffusion, and flow dynamics of fluorescent proteins in your living samples in a single, easy measurement. Develop a more in-depth profile of the molecules in your current experiments, from cell cultures to organoids to whole organisms.

zeiss.com/dynamics-profiler



ZEISS LSM 980 with Airyscan 2

Your Unique Confocal Experience for Fast and Gentle Multiplex Imaging

To analyze life with as little disturbance as possible, you must use low labeling density for your biological models. This requires excellent imaging performance combined with low phototoxicity and high speed. LSM 980 is optimized for simultaneous spectral detection of multiple weak labels with the highest light efficiency.

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