

# Lambda Square Mapping and FLIM

## Explore photonic landscapes with the Leica TCS SP5 X

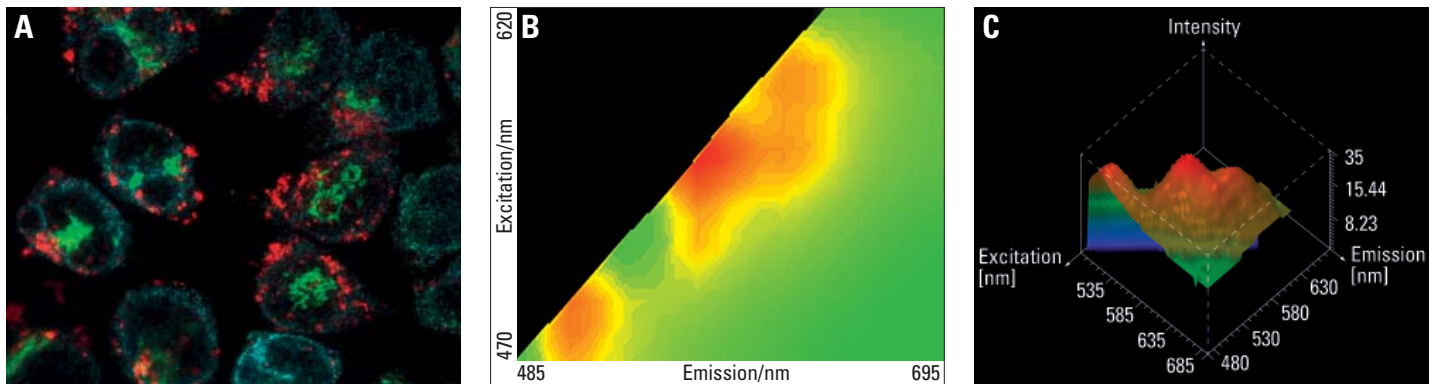
- Full spectral analysis of images and lifetime measurements
- User guidance by interactive experiment definition
- “Dye Hunting”: screening for fluorescent proteins and discovering new dyes

Living up to Life

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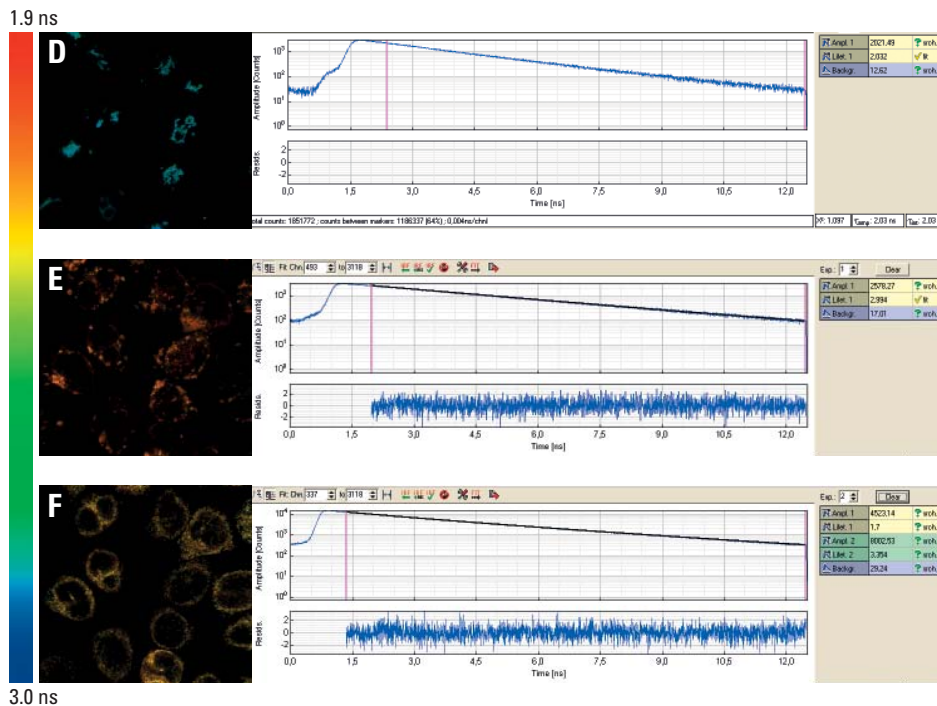
$\lambda^2$  mapping identifies each fluorescent molecule by its characteristic excitation-emission spectrum which is acquired with the tunable white light laser. Using this information, the Leica TCS SP5 X imaging settings can be optimized to maximize fluor-

escence emission and minimize cross talk, cross excitation, and autofluorescence. Your research benefits from the highest image contrast and data quality. New dyes or fluorescent proteins can be discovered and characterized.



Sample: fixed cells with triple staining (A): GalNacT2\_GFP (golgi), LAMP-546 (endosomes), Calnexin 594 (ER). Corresponding fluorescence peaks are visible in a logarithmic scaled  $\lambda^2$  plot (B, C).

Below: the FLIM measurements at different excitation wavelengths (D: 486 nm, E: 542 nm, F: 594 nm) show specific fluorescence lifetimes of the three labels. Sample: courtesy of Matthias Weiss, Cellular Biophysics Group, Bioquant, Heidelberg, Germany.



**FLIM:** The fluorescence lifetime is a characteristic property of each fluorophore. Also, it provides information about the direct environment of a molecule, like local pH or binding to another molecule. The combination of spectral and lifetime information yields the maximum information available about a molecule and enhances the reliability of data interpretation.