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Focal Adhesion Axial Topography by the Z-Phasor Approach in Confocal Microscopy

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Usually, image data acquired with a camera is analyzed by correlating the fluorescence time trace captured in each pixel followed by fitting a function modeling the dynamic process. Yet, with ~2^{16} data points at hand, manual data evaluation is impossible while automatic fitting procedures are prone to errors because they rely on careful adjustment of start parameters and constrains. In particular, a fit to correlation spectroscopy data from regions containing static molecules or no molecules at all produces inconclusive results.

In fluorescence lifetime imaging (FLIM), the phasor approach is a popular method to obtain a graphical representation of the spatial distribution of lifetimes [1]. Phasor-based analysis is fast and does not require any fitting of the data. We applied the phasor method to camera-based correlation spectroscopy resulting in a map of molecular diffusion. This map can be overlaid with the intensity image to quickly identify spatial variations of the diffusion of fluorescently labeled biomolecules within living cells.

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[1] M. Digman et al. *The phasor approach to fluorescence lifetime imaging analysis.* Biophysical Journal 94 (2008), L14-L16.

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Diffusion Mapping in Living Cells using Camera-Based Correlation Spectroscopy and Phasor Analysis

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Image correlation spectroscopy (ICS) is a powerful tool to study dynamics in living cells and tissues. However, when using a single point detector, the mapping of molecular movement is either slow or limited to a small field of view due to the sequential, point-by-point data acquisition. With an area detector such as a camera on the other hand, molecular motion can be captured simultaneously for each image pixel, allowing a fast mapping of the entire structure under study.