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

Journal
Biophysical Journal, 106(2)

ISSN
0006-3495

Authors
Hedde, Per Niklas
Gratton, Enrico

Publication Date
2014

DOI
10.1016/j.bpj.2013.11.1189

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.

Work supported in part by NIH grants P50 GM076516 and P41 GM103540.

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

1020-Pos Board B775

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

Per Niklas Hedde, Enrico Gratton.
Laboratory for Fluorescence Dynamics, Biomedical Engineering Department, University of California Irvine, Irvine, CA, USA.

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.