

SIMULTANEOUS SPECTRAL ACQUISITION AND LINEAR UNMIXING ALLOWS THE ANALYSIS OF COMPLEX DYNAMICS OF CELLULAR STRUCTURES

Jens Rietdorf, Emmanuel G. Reynaud, Timo Zimmermann, Kota Miura and Rainer Pepperkok

**Advanced Light Microscopy Facility
Cell Biology and Biophysics Department
European Molecular Biology Laboratory
Meyerohofstr. 1, 69117 Heidelberg, Germany
E-mail: rietdorf@embl.de**

KEYWORDS: Multi-dimensional live cell imaging, spectral imaging, linear unmixing, cytoplasmic crowding, intracellular membrane transport.

Eukaryotic cells consist of numerous structural and functional domains and compartments that can be distinguished microscopically by means of specific fluorescent labels. Many of these structures are characterised by a steady dynamic flux, exchange of material and movement. Because of the network-like interdependence of several signal transduction or transport pathways, it would be highly desirable to observe a large number of these structures simultaneously.

The simultaneous observation and analysis of multiple dynamic events inside living samples has however so far been hindered in several general respects: (1) Extensive illumination harms the sample (2) Available fluorophores have overlapping emissions which can not be efficiently separated (3) Available instruments only allow to acquire a very limited number of channels at the same time.

A combination of state-of-the-art spectral microscopy, light-efficient separation of fluorescence emission from six fluorophores by linear unmixing [1] together with computational methods to quantify movements were used to analyse dynamic events at an unprecedentedly high level of complexity. We have applied these techniques in a systematic approach to characterise the dynamics of cellular substructures like the Endoplasmic Reticulum.

[1] Zimmermann T, Rietdorf J, Pepperkok R. Spectral imaging and its applications in live cell microscopy. *FEBS Lett.* **546**(1):87-92. Review. 2003