VISUALISATION OF MULTIPLE COUPLED SIGNALLING EVENTS IN A SINGLE CELL BY FLUORESCENCE LIFETIME PATTERN ANALYSIS.

Anastasia Loman1,*, Ingo Gregor3,*, René Ebrecht1, Jörg Enderlein3, Fred S. Wouters1, and Gertrude Bunt1,2

1 Molecular and Cellular Systems, Neuro- and Sensory Physiology, University Medicine Göttingen, Humboldtallee 23, D-37073 Göttingen, Germany
2 DFG Excellence Cluster 171 “Microscopy at the Nanometer Range”
3 3rd Institute of Physics, Georg-August-University, Friedrich-Hund-Platz 1, D-37077 Göttingen, Germany
* equal contribution
E-mail: gbunt@gwdg.de

KEY WORDS: multiplexing, lifetime algorithms, pattern analysis, FLIM, coupled signalling events, FAK activation, single cell detection.

The cellular signalling network consists of an intricate protein interaction matrix with spatial and temporal resolution. Analysis of the hierarchy and connectivity – identifying integrating nodes and con/di-vergent connections – of signalling events at the (sub)cellular level will provide us with invaluable insight into causal relationships and, with this, into cellular functioning in physiology and disease. This information can only be obtained by the simultaneous visualization of multiple protein activities in a single cell. Others have explored the use of spectral or spatial discrimination for multiplexed analyses. We present a method whereby multiple optical bioassays can be combined by virtue of their fluorescence lifetime contrast. Their quantitative unmixing is based on fluorescence decay pattern analysis.

By the selection of fluorophores with optimally suited lifetime contrast, we are able to decompose the signals of three fluorophores within one spectral window, permitting a total of nine fluorophores over three windows. The linear unmixing of lifetime decay patterns can be performed blind or referenced and can be used with multi-exponential decays. Most importantly, our approach allows the discrimination of events that spatially co-localize. We applied our method to detect three connected and spatially overlapping protein activities using FRET-based sensors and ratiometric detection based on antibodies: (i) an activation-associated conformational change of focal adhesion kinase, (ii) its phosphorylation and (iii) the phosphorylation of its effector paxillin. Our approach is ideally suited for the detection of endogenous protein activities, avoiding the need to introduce multiple genetically-encoded sensors into cells which can perturb its physiology of the cell, and permitting its facile application for diagnostic screening on pathological tissue specimens.