Spatio-Temporal Interrogation of Signaling Networks Using Biosensor Imaging and Optogenetics

O.P. Pertz[1]

[1] Institute of Cell Biology, University of Bern, Switzerland.

Abstract

Genetically encoded biosensors that report on signaling dynamics in single living cells have revolutionized our understanding of signaling networks. Here, I present novel technological implementations that showcase the power and promise of biosensor imaging. Previously, we showed that dynamically perturbing signaling networks at the exact timescale at which they fluctuate provides unique information about their circuitry. To unlock the full potential of this approach, I describe a genetic circuit comprising a receptor tyrosine kinase (RTK) optogenetic actuator and a spectrally orthogonal biosensor that reports on the activity of the MAPK ERK. This enables us to challenge cells with highly standardized temporal RTK inputs and measure the resulting ERK signaling outputs. Coupled with microscope automation and automated image analysis, this allows for high experimental throughput in studying single-cell signaling dynamics. This leads to large amountsof biosensor time-series biosdatasets that escape visual inspection by humans. To enable scalable dataanalysis, we present a machine learning approach that enables data-driven analysis of single-cell biosensor timeseries. We showcase how this approach allowed us to analyze large datasets of single-cell ERK dynamics in response to several perturbations, and provide new insight in how the MAPK network is wired to control fate decisions. Additionally, I report on a real-time feedback microscopy platform dedicated to optogenetics that provides on-the-fly image analysis and subsequent automated optogenetic illumination with any desired spatio-temporal light input. I showcase how this platform can be used to study spatio-temporal Rho GTPase and MAPK/ERK network circuitry.