Hessisches Kompetenzzentrum für Hochleistungsrechnen

Computer-Aided Analysis of Live-Cell Microscopy Datasets

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Project Term
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Clusters
Lichtenberg II Cluster Darmstadt

Software
MATLAB, Python

Institute
Systems Biology of the Stress Response

University
Technische Universität Darmstadt

Introduction

During development and adult homeostasis, cells in our bodies need to constantly integrate internal and external information to adjust their physiology and chose appropriate cell fates. To understand cellular signal processing, we need to reveal how the underlying molecular networks function dynamically in individual living cells. Live-cell time-lapse microscopy in combination with fluorescent reporter cell lines allows to follow molecular processes with unrivaled temporal and spatial resolution and provides the necessary quantitative data to gain a mechanistic understanding of cellular signaling. However, to quantify the resulting large data sets on the single cell level, sophisticated image analysis algorithms are required. To allow timely data-analysis, computer-aided segmentation, object tracking and classification is scaled to the parallel computing infrastructure of the Lichtenberg cluster.

Methods

Using automated fluorescent microscopy, we acquire high-resolution images of reporter cell lines from 60 – 300 field of views in up to four channels for periods of 24 – 96h with a time resolution of 3 - 20min. Individual cells are tracked throughout the duration of the experiment using custom-written Matlab scripts. In brief, we first apply flat field correction and background subtraction to raw images before segmenting individual nuclei from nuclear marker images using adaptive thresholding and seeded watershed algorithms. Segmented objects are then assigned to corresponding objects in following images using point set registration and a greedy match

algorithm. We finally track cells in forward or backward direction from the first to the last time point and quantify various features of the fluorescence signals. This typically results in ten thousand of single cell trajectories for each experiment.

Results

In previous funding periods, we migrated our image analysis framework to the Lichtenberg Cluster and established corresponding workflows for data transfer. During this period, we continued to optimize our scripts and adjusted them to current Matlab versions (R2024a). Meanwhile, we routinely used the Lichtenberg Cluster for data analysis in the context of ongoing research projects. For example, we combined quantitative fluorescent live-cell microscopy, computational data analysis and mathematical modeling to analyze pulsatile accumulation of p53 protein upon induction of DNA double strand breaks. Initial studies indicated that these pulses are generated by an excitable network comprising positive and negative feedback, resulting in uniform amplitude and duration. The strength of the insult is mainly encoded in the number of pulses, like a digital signaling system. We now investigated how the molecular network regulating p53 dynamics shapes its response to acute and sustained stress. Our results indicate that the damage regulated kinase ATM is crucial for initiating the p53 response, while the check point kinase CHK2 sustains the response in the presence of persisting damage by moving the negative feedback between p53 and its ubiquitin ligase in a stable limit cycle. This leads to sustained p53 oscillations, whose duration depends on the initial amount of activated Chk2. Taken together, our systems biology approach provides evidence how specific dynamics of the tumor suppressor p53 enable individual cells to appropriately respond to varying type and doses of genotoxic stress and thereby prevent tumorigenesis.

Discussion

Using the Lichtenberg Cluster for our image analysis framework allows us to efficiently process large amounts of imaging data and significantly expands the scope of our experiments.

Publications

Vigliotti, F., Engelmann, N., Sinzger-D'Angelo, M., Koeppl, H., & Loewer, A. (2025): Decoding stimulus-specific regulation of promoter activity of p53 target genes. Frontiers in Cell and Developmental Biology, 13. https://doi.org/10.3389/fcell.2025.1603603

Hartmann, Laura, et al. (2025): Transcriptional regulators ensuring specific gene expression and decision-making at high TGFβ doses. Life Science Alliance 8.1.

Reference

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