Computer-Aided Analysis of Time-Lapse Microscopy Data II

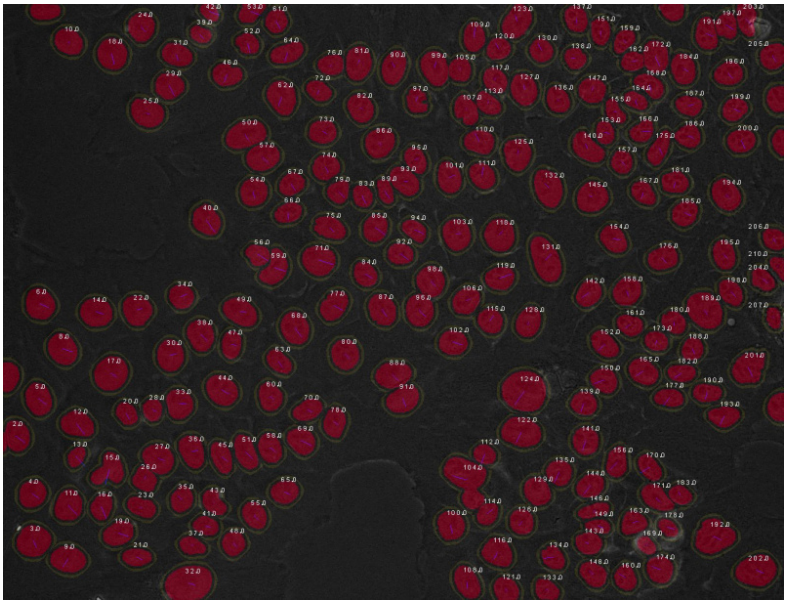


Figure 1: Image segmentation and object identification of human breast epithelial cells.

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Project Term
2022 - 2023

Clusters
Lichtenberg II Cluster Darmstadt

Software
MATLAB

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 thousands of single cell trajectories for each experiment.

Results

In the last project period, we migrated our image analysis framework to the Lichtenberg Cluster, established corresponding protocols for data transfer to the file systems of the cluster and optimized workflows. We now routinely use the cluster resources in our research projects. For example, we investigated how the molecular interactions regulating the fate of individual cells evolve during episodes of continuous stress using the tumor suppressor p53 as a paradigm. In response to DNA damage, p53 shows pulsatile accumulations that lasts from hours to days. Combining single-cell analysis and mathematical modelling, we revealed differences in the response to acute and sustained stress, as the regulatory network shifts from an excitable state to a stable limit cycle. Interestingly, the different regimes of the network are regulated by distinct regulators. Taken together, we provided evidence that differential regulation of p53 by upstream kinases allows cells to respond reliably both to acute and persisting DNA breaks. In other projects, we quantified the DNA damage response upon low dose radiation and investigated the dynamics of SMAD signaling in endothelial and epithelial cells.

Discussion

The resources of the Lichtenberg cluster allow us to scale up the computational analysis of time-resolved live-cell imaging data and therefore enable us to process more and higher resolved data regarding dynamic information processing in individual cells.

Publications

Loewer, A. Keeping the guardian alert: different upstream regulators enable p53 to differentiate between acute and sustained DNA damage in individual cells, German-French DNA repair meeting, Mainz, Germany, September 13-15 (2023)

Loewer, A. Keeping the guardian alert: how p53 differentiates between acute and sustained DNA damage in individual cells, Hohenwart, Germany, September 18-20 (2023)

Last Update: 2024-01-30 10:55