Fluorescence Correlation Spectroscopy as Tool for High-Content-Screening in Yeast (HCS-FCS)

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We developed tools to do automated auto- and cross-correlation Fluorescence Correlation Spectroscopic (FCS) measurements on thousands of individual living cells. We take an image of each cell we measure. We used this system to investigate the local concentrations and diffusion properties proteome-wide in the budding yeast S. cerevisiae using auto-correlation FCS. For this screen we took over 200,500 measurements on more than 50,000 individual cells for 4082 clones of eGFP tagged open reading frames (ORF). We extended the set-up to do interaction studies based on two-color cross-correlation FCS (FCCS). We use homologous recombination to replace ORFs in the eGFP clone library with mCherry tagged versions. The interaction data we measured is in excellent agreement with manual FCCS and biochemical results.

All high-content FCS screens (HCS-FCS) were performed in 96 well plates with optical glass bottom. The system is based on an extended Carl Zeiss fluorescence correlation spectrometer ConfoCor 3 attached to a confocal microscope LSM 510. We developed image-processing software to control these hardware components. The confocal microscope takes overview images and the algorithm searches for and detects single cells. At each cell we position a laser beam at a well-defined position within the cell and record the fluctuation signal. All data is stored and organized in a database based on the open source Open Microscopy platform. Data analysis is performed in the image processing language IDL and the open source statistical software package R.

To minimize spectral cross-talk in cross-correlation measurements we switch between the two excitation lasers with a frequency of about 10 kHz. For data evaluation we extract the actual frequency and phase from the raw data using a Fourier transformation.

For the automatic data analysis, we developed algorithms based on machine learning to classify the images of the measured cells to include only measurements that where taken on healthy, non-moving, and in-focus cells. We developed a statistical approach to exclude erroneous data. Common reasons are cells which move during measurement, aggregates drifting through the confocal volume, and bleaching of immobile dyes. In addition, to allow for automation, this approach has the advantage to eliminate any human bias. We fit different fluctuation models to the data and select the most appropriate one using Akaike’s ‘An Information Criterion’ (AIC).