AUTOMATED FLUORESCENCE-CORRELATION SPECTROSCOPY FOR HIGH-CONTENT-SCREENING (HCS-FCS)

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We will present a pipeline for automated fluorescence-correlation spectroscopy (FCS). This setup allows us to increase the signal-to-noise for live cell FCS by measuring large numbers of cells. We used FCS to measure local concentrations and diffusion properties for more than 4000 different proteins in individual yeast cells (Saccharomyces cerevisiae). We applied fluorescence-crosscorrelation spectroscopy (FCCS) to investigate the interaction of 18 proteins associated with the Skp1-Cul1-F box (SCF) ubiquitin ligase complex. The SCF complex ubiquinates proteins and marks them for digestion by the proteasome.

Our screens are based on clones derived from the yeast-GFP library developed by Huh et al. A robotic SGA procedure allowed us to include mCherry into a subset of yeast clones for two-color FCCS measurements. A modified, commercial FCS system (LSM510-ConfoCor3, Carl Zeiss Jena, Germany) acquired images of individual yeast cells grown in glass-bottom 96-well plates. For each automatically selected yeast-cell, our system acquired a one or two-color fluctuation curve.

We wanted to make sure to include only healthy cells into our analysis. Therefore, we developed a machine learning approach for the selection process. We applied a wavelet filter to the fluctuation curves to correct for bleaching artifacts. We then calculated auto- and cross-correlation curves and fitted different diffusion models to the data to extract quantitative information. For each model, we calculated Akaike’s Information Criterion (AIC) to select for the most appropriate description of the data.