

TOOLS FOR GENOME WIDE FLUCTUATION ANALYSIS OF *S. CEREVISIAE*

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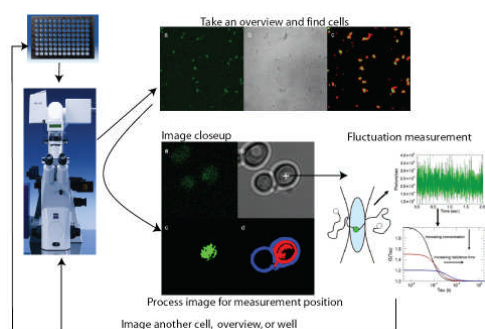


Figure 1: The automated measurement process. For details, see text.

To understand the function of yeast proteins, we started a genome-wide investigation into their localization, concentration, diffusion properties, oligomeric state, and interactions with other partners. As measurement tools we choose Fluorescence Correlation Spectroscopy (FCS[1] and FCCS[2]), Photon Counting Histograms (PCH)[3, 4], and confocal imaging using Avalanche Photo Detectors (APD). Our instrumentation is based on a

commercial confocal microscope (LSM 510 META, Carl Zeiss Jena, Germany) equipped with a fluorescence correlation spectrometer (ConfoCor 3, Carl Zeiss Jena, Germany). We developed software tools written in IDL (ITT Visual Information Solutions, Boulder, CO) to detect single yeast cells and control the data acquisition. We use a GFP (Green Fluorescence Protein) clone collection of *Saccharomyces cerevisiae* tagged open reading frames generated at UCSF[5].

We use IDL to analyze the fluctuation data and to determine the localization of the proteins based on our imaging results. To organize and query the data we use the Open Microscopy Environment [6].

- [1] E. L. Elson and D. Magde, "Fluorescence correlation spectroscopy. I. Conceptual basis and theory," *Biopolymers*, vol. 13, pp. 1-27, 1974.
- [2] P. Schwille, F. Meyer-Almes, and R. Rigler, "Dual-color fluorescence cross-correlation spectroscopy for multicomponent diffusional analysis in solution," *Biophys. J.*, vol. 72, pp. 1878-1886, April 1, 1997.
- [3] Y. Chen, J. D. Müller, P. T. C. So, and E. Gratton, "The photon counting histogram in fluorescence fluctuation spectroscopy," *Biophysical Journal*, vol. 77, pp. 553-567, 1999.
- [4] P. Kask, K. Palo, D. Ullmann, and K. Gall, "Fluorescence-intensity distribution analysis and its application in biomolecular detection technology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, pp. 13756-13761, 1999.
- [5] W. K. Huh, J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, and E. K. O'Shea, "Global analysis of protein localization in budding yeast," *Nature*, vol. 425, pp. 686-691, 2003.
- [6] J. R. Swedlow, I. Goldberg, E. Brauner, and P. K. Sorger, "Informatics and quantitative analysis in biological imaging," *Science*, vol. 300, pp. 100-102, 2003.