The ACQUIFER Imaging Machine: An automated microscopy platform for high-throughput screening of budding yeast

The budding yeast *Saccharomyces cerevisiae*, an eukaryotic microorganism, is a powerful model organism to address biomedical research questions on the genome-scale using growth or biochemical assays. This is complemented by large-scale imaging screens using automated high-throughput microscopy to visualize fluorescent reporter localizations. This allows monitoring the full yeast proteome via GFP fusion proteins or any phenotype that can be followed by a fluorescent marker. Due to the small cell sizes, photosensitivity and non-adherence of yeast cells, these high throughput screening assays demand advanced automated imaging platforms that are capable of robustly and reproducibly acquiring high-resolution datasets for visualization and scoring of cellular and sub-cellular phenotypes.

In **yeast high-resolution screening**, liquid cell cultures are usually dispensed in coated 384-well glass-bottom plates using automated liquid handling techniques. Plates are then manually or robotically loaded on automated microscopy platforms, and subsequently imaged to score cellular or genetic events. **Live-cell imaging** in yeast requires stable temperature conditions to observe cellular processes under optimal environmental conditions. This is especially crucial when growth, proliferation, protein trafficking or other dynamic parameters are addressed in **timelapse experiments**. The microorganismal nature of yeast demands **high magnification** objectives to sufficiently capture and visualize cellular phenotypes. The often weak expression of fluorescencent reporter gene fusions under the control of their endogenous promoter require ahigh numerical apertures to analyse the spatio-temporal activity of signals. However, high magnification and high numerical apertures result in small fields of view and very narrow depth-of-focus, thus **precise XY-positioning** and **Z-autofocusing** are of vital importance.
The **ACQUIFER Imaging Machine** constitutes a benchtop sized robotic widefield microscopy platform, fully compatible with advanced yeast high throughput screening assays. Its **robotic incubation lid** renders it compatible with automatic plate handling and provides stable temperature conditions for long term observations and incubation. A **stationary sample holder** avoids the application of any movement generated force to the sample - instead the entire optical block moves underneath the microplate. High numerical aperture objectives, in combination with stable LED-based illumination and a state-of-the-art sCMOS camera, ensure **sufficient resolution, intensity and sensitivity** for yeast fluorescent imaging. Dedicated **yeast software autofocus** routines for bright-field images and an infrared **laser hardware autofocus** ensure precise in-focus capture of yeast cells while keeping phototoxicity and photobleaching to a minimum. Finally, **data handling and image processing** capacity is directly integrated via the **ACQUIFER HIVE** platform.

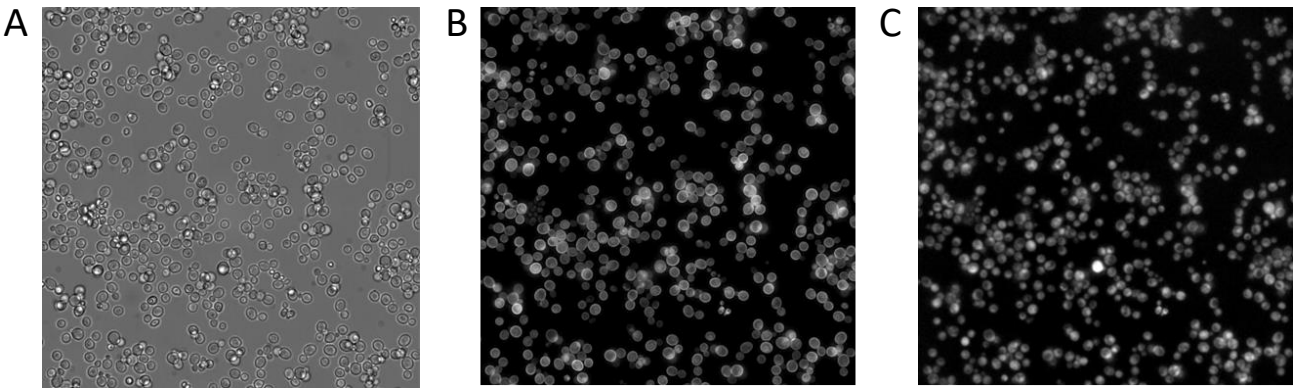

Figure 1: Automated high throughput screening of yeast. 384-well plates containing yeast liquid culture are imaged on the ACQUIFER Imaging Machine. Illustrative examples of a multidimensional image acquisition of (A) bright-field channel, (B) 470 nm channel indicating localization of a GFP-fusion protein and (C) 595 nm channel showing mCherry expression highlighting overall cellular morphology are shown. Images were taken using a 40x NA 0.65 objective.

The ACQUIFER Imaging Machine is a comprehensive automated microscopy platform enabling advanced automated microscopy and high-throughput screening of *Saccharomyces cerevisiae*

ACQUIFER Imaging Machine features:

- Motorized lid for automated microplate loading
- Integrated incubation for stable and precise temperature control
- Sample centred approach – no movement of samples
- Automated high-resolution imaging of sensitive or weak fluorescent reporters
- Robust and precise lighting and XYZ-movement control
- Custom yeast software autofocus
- Infrared laser hardware autofocus
- Easy-to-use software and graphical user interface
- Integrated data-handling and image processing hardware

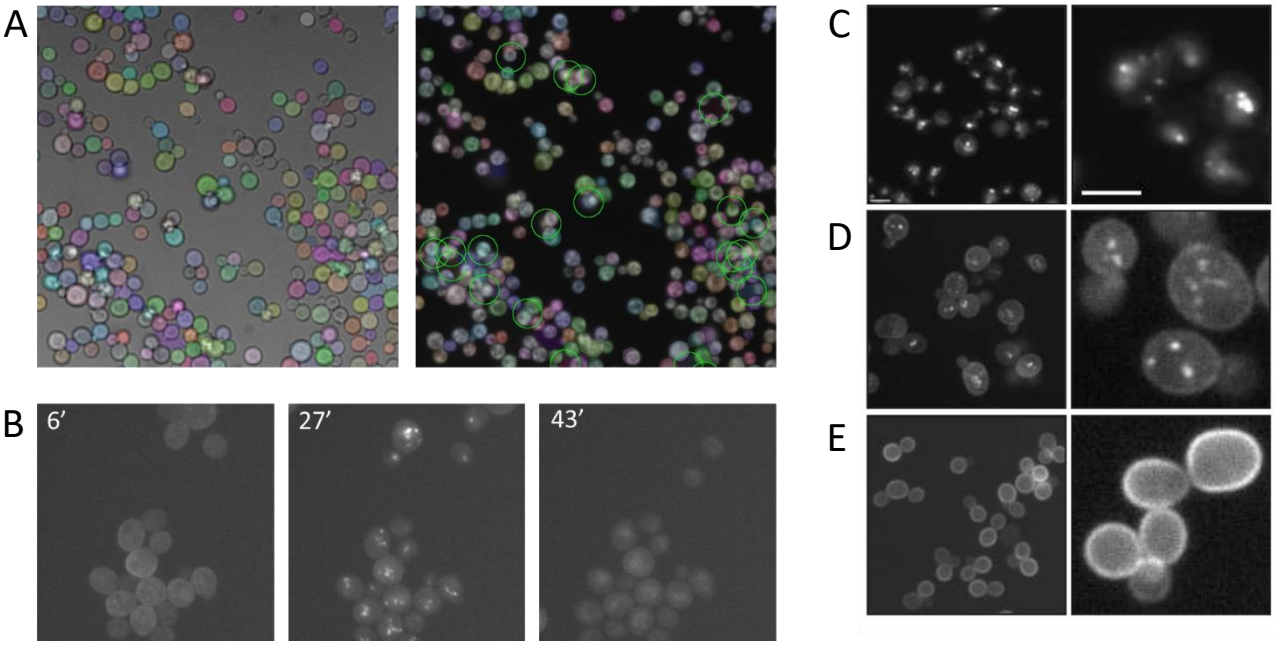

Figure 2: Application examples of yeast high throughput screening on the ACQUIFER Imaging Machine. (A) High content screening datasets allow automatic processing using image analysis workflows providing single-cell statistics of cellular features in the order of 100s of cells or reporter gene expression status. Connected component analysis visualizations after single cell segmentation are shown. Each single cell detected is indicated by a separate colour. Additionally, automated outlier removal (e.g. dead cells) is highlighted by green circles. (B) Automated time-lapse analysis of yeast cells to analyse intracellular protein trafficking. Yeast cells at 6 min, 27 min and 43 min after nutrient withdrawal are shown allowing visualization and scoring of the trafficking of a GFP-fusion protein. (C-E) Examples of automated imaging of yeast to analyse localization of reporter-fusion proteins to intracellular structures (C-D) or the plasma membrane (E). Scale bars in C-E are 5 µm. Images courtesy of Anne Clancy and Prof Blanche Schwappach, Universitätsmedizin Göttingen and Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany.

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