Application Brief

Live cell imaging of yeast with the CellASIC® platform reveals effect of the extracellular milieu.

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The benefits of studying live, single yeast cells.

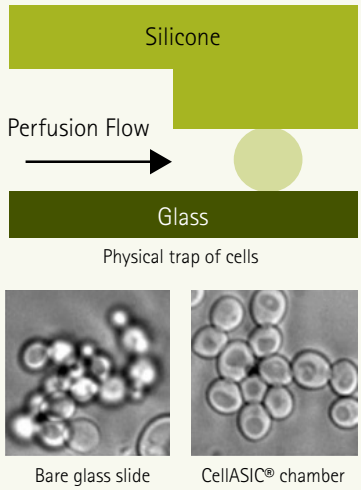
Yeast cells are an important class of model organisms for the study of eukaryotic cell biology. Yeast cells are used in bioreactors, food preparation, synthetic biology, infectious disease research, and other applications, because they are easy to culture, can be genetically manipulated, and are homologous, in many ways, to human cells. Moreover, using live, single-yeast-cell analysis, yeast researchers have been able to obtain extremely detailed information, such as such as cell-to-cell variation, dynamic response profiles, intergenerational relationships, spatial information, and morphological features.

Advances in high resolution microscopy enable quantification of individual yeast cells, but traditional culture chambers do not provide optimal conditions for tracking cells over time. Because most yeast cells are small (~4 µm) and nonadherent, live cell imaging experiments are difficult with conventional methods. Yeast cells move so quickly out of a field that it is usually challenging to monitor the same group of cells over time.

With microfluidics, yeast cells are trapped and easy to monitor.

Merck Millipore has developed the CellASIC® ONIX platform (Cat. No. EV262) for spatially localizing yeast to a single monolayer for long-term, high quality live cell microscopy. The microfluidic chambers inside the CellASIC® ONIX Y04C cell culture plate traps cells using an elastic ceiling (3.5-4.5 µm height) without preventing perfusion flow or hindering cell growth.

Six upstream fluidic channels allow controlled exposure of the cells to different solutions during live imaging. The cells are in contact with a #1.5 thickness (170 µm) optical glass surface, enabling high quality imaging using an inverted microscope.



Trapping chamber keeps cells in focus

Track yeast over multiple generations for 12+ hours while maintaining a single focal plane.

The microfabricated chamber gently holds cells against the glass viewing surface to maintain a single focal plane during perfusion experiments.

Control the yeast cell microenvironment without interrupting microscopy/imaging.

A key feature of the CellASIC® ONIX Y04C plate design is that solutions can be changed during live cell imaging without perturbing the plate or microscope. This enables tracking of cell responses to changing solution environments. The plate allows six different solutions to be switched during the course of the experiment.

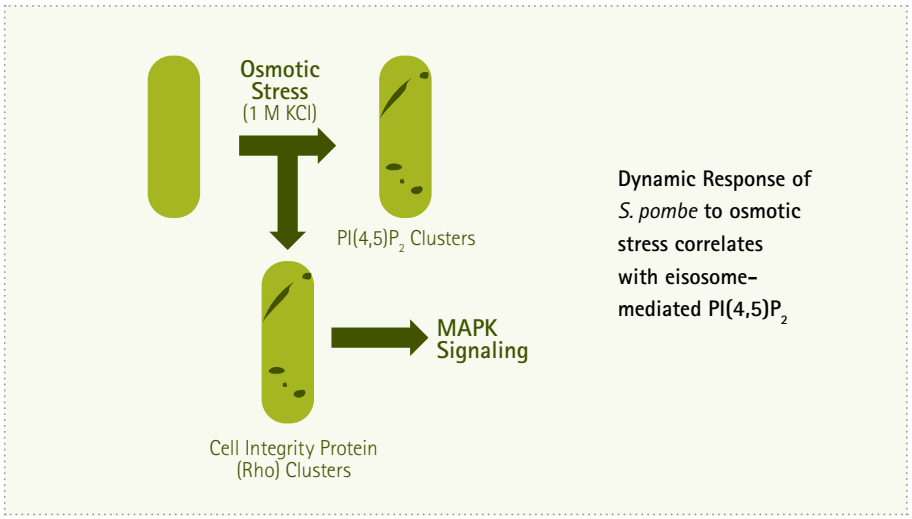
The favorable cell culture environment in the CellASIC® ONIX Y04C chamber enables long-term maintenance of yeast under precisely controlled conditions. Since the cells are trapped in x,y,z space, it is easy to track cell responses over time on the same group of cells. This allows the collection of kinetic response data on live cells, which is not possible with other approaches. Moreover, because the cells are prevented from moving, intracellular dynamics (changes that occur with respect to time) can also be monitored with high resolution. Cell growth in the trap chamber was equivalent to expected values, indicating there is no detrimental effect of the microfluidic culture configuration (data not shown).

Selected publications by CellASIC® ONIX users in yeast cell research

Over the last eight years, many yeast researchers have cited the CellASIC® ONIX system in their publications[1-8, others]. Their discoveries have yielded insights into previously mystifying cellular processes, a few of which we highlight below:

Stressed out cells.

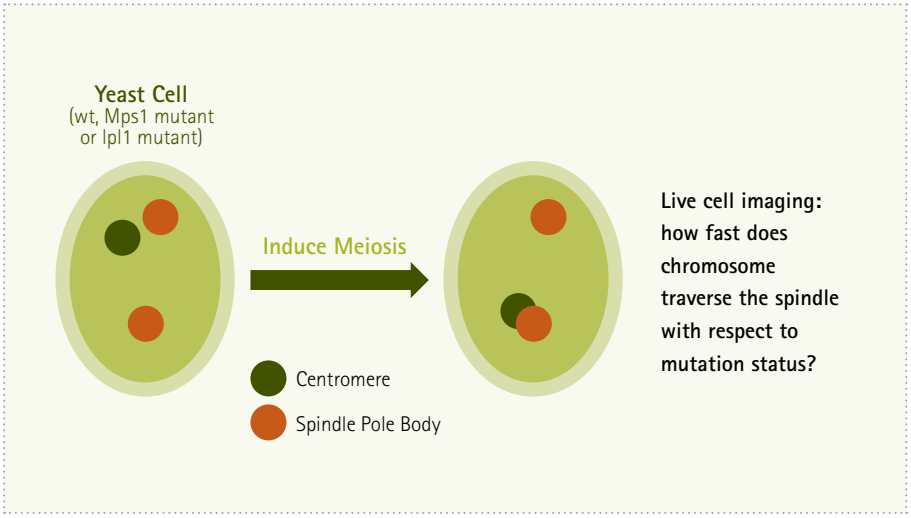
Kabeche and colleagues, in a very recent study[1], used live yeast cell analysis to uncover the function of eisosomes, which are protein structures that caused the plasma membrane to fold in on itself. Because the researchers were able to deliver osmotic shock to live cells and watch them respond in real time, they discovered that these eisosomes appeared to organize lipids in the membrane, causing signaling molecules to cluster in response to the environmental stress.



Dynamic Response of *S. pombe* to osmotic stress correlates with eisosome-mediated PI(4,5)P_2

Dynamic chromosome segregation.

For proper segregation, paired chromosomes must attach to opposite spindle poles in a precise fashion, mediated by essential kinases. But how do these kinases act, and in what order? In a 2013 *Science* paper, Meyer and colleagues reported using the CellASIC® ONIX platform to monitor how fast chromosomes traveled across the spindle with respect to mutation status of Mps1 and Ipl1 kinases[3]. They were able to determine the sequence in which these two kinases worked to ensure accurate chromosome segregation.



Conclusion

The CellASIC® ONIX Microfluidic Plate is a perfusion chamber specifically designed to trap yeast cells in a single focal plane for imaging without limiting solution exchange or cell growth properties.

This design has been demonstrated for monitoring *S. cerevisiae* and *S. pombe* for long-term culture, fluorescence quantification, solution exchange response, and time-varying inputs. Further, the ease of use, flexibility, and accessibility of this advanced technology platform should prove beneficial to a wide range of yeast cell biology applications*.

* Dynamic processes in yeast cells revealed by the CellASIC® ONIX Platform:

- Cell response to media change
- Induction of cell cycle events
- GFP-linked nuclear trafficking
- Live cell microscopy of mitosis
- Starvation and recovery
- Gene expression
- Protein localization
- Mitochondrial inheritance
- Phase contrast, fluorescence, brightfield, confocal, TIRF and DIC microscopy

References

1. Eisosomes regulate PI(4,5)P2 cortical clusters and MAP kinase signaling upon osmotic stress. Kabeche R, Madrid M, Cansado J, Moseley JB. J Biol Chem (2015).
2. Feed-forward regulation ensures stability and rapid reversibility of a cellular state. Doncic A, Skotheim JM. Molecular Cell, 50 (2013).
3. Mps1 and Ipl1/Aurora B act sequentially to correctly orient chromosomes on the meiotic spindle of budding yeast. Meyer R, Kim S, Obeso D, Straight P, Winey M, Dawson D. Science, 339 (2013).
4. An algorithm to automate yeast segmentation and tracking. Doncic, Andreas, et al. PLoS ONE, 8 (2013).
5. Mitochondrial network size scaling in budding yeast. Rafelski S et al. Science, 338 (2012).
6. Continued DNA synthesis in replication checkpoint mutants leads to fork collapse. Sabatinos SA, Green MD, Forsburg SL. Molecular and Cellular Biology, 32 (2012).
7. Bacterial virulence proteins as tools to rewire kinase pathways in yeast and immune cells. Wei P, Wong W, Park J, Corcoran E, Peisajovich S, Onuffer J, Weiss A, Lim W. Nature, 488 (2012).
8. Epigenetic and conventional regulation is distributed among activators of FLO11 allowing tuning of population-level heterogeneity in its expression. Octavio LM, Gedeon K, Maheshri N. PLoS Genet. (2009).

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