Application Note

Poseidon Select™

Scanning Transmission Electron Microscopy of Live Yeast Cells in Liquid

Reference: Peckys, Diana B., P. Mazur, et al. (2011). "Fully Hydrated Yeast Cells Imaged with Electron Microscopy." Biophysical Journal 100(10): 2522-2529.

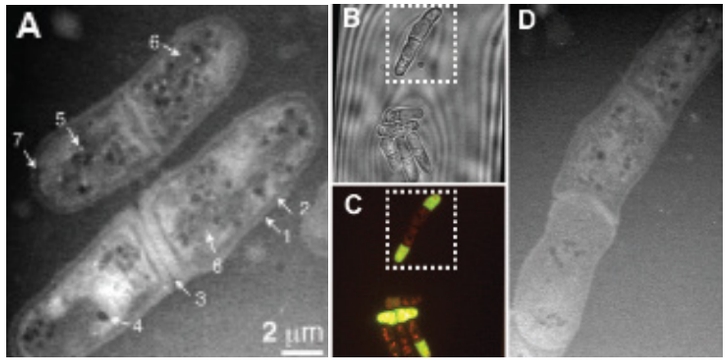
Introduction

A key complicating factor in whole cell electron microscopy is the need to embed and section samples prior to imaging, which complicates experimental procedures and may introduce artifacts.

The Poseidon *in situ* system enables fully hydrated samples to be imaged with electron microscopy. In this experiment, images of live yeast cells were recorded with scanning transmission electron microscopy (STEM) using the Poseidon system. Experiment Live wild type and a septin mutant (spn3 Δ) strain of *schizosaccaromyces pombe* (*s. pombe*) yeast cells were incubated for 60 minutes in 10 µM of live/dead indicator dye (FUN-1). The Poseidon system holder was loaded by dispensing a droplet of *s. pombe* cell suspension on the lower E-chip™. A second E-chip was placed on top and the chamber was sealed. Fluorescent images were recorded prior to and after electron microscopy. STEM images were recorded using a Philips/FEI CM200 TEM/STEM operating at 200kV. The liquid thickness in the imaged regions was measured to be 3 ± 1 µm. Images of the wild type and mutant strains were recorded with an electron dose of 22 e-/nm^2.

Discussion

The STEM image of wild-type yeast cells is shown in Figure A. Internal structures such as the cell wall (1), the primary septum (2), the secondary septum (3), a cell membrane invagination (4), lipid droplet (5), a peroxisome (6), an unclassified vesicle (7), and a gold nanoparticle are all visible.



Correlative light and electron microscopy (CLEM) was also demonstrated. Figure B shows a phase contrast light microscopy image of the mutated yeast cells which contained the live/dead indicator dye. The corresponding fluorescence image is shown in Figure C. Living cells exhibit a punctuated red fluorescence, while dead cells exhibit a bright green fluorescence. Thus, the cells exhibiting red fluorescence were known to be alive at the onset of STEM imaging. The corresponding STEM image of the cells in the dashed box is shown in Figure D. A resolution of 32 ± 8 nm was obtained, which effectively provides a bridge between the resolution obtained with light microscopy and traditional biological TEM techniques.

Applications

The Poseidon liquid *in situ* system enables imaging of live cells in a fully hydrated state, using electron microscopy. The resolution obtained in this study was sufficient to resolve the ultrastructural details of pristine yeast cells without freezing or plastic embedment. Contact us to discuss the full range of capabilities of Poseidon Select. We can be reached at (919) 377-0800 or contact@protochips.com.

Protochips