MICROSCOPY

Etch-a-cell

A milling technique affords researchers a high-resolution glimpse deep into the cell using cryoelectron tomography.

Cryoelectron tomography, or cryoET, is one of the best technologies for examining the supramolecular organization within cells in a 'close-to-life' state—that is, a frozen but hydrated state. But the resolution constraints of transmission electron microscopy (TEM) have limited cryoET to bacteria or isolated organelles. To look deep into thick eukaryotic cells, cryosectioning may be performed but comes with a high risk of distorting cellular features. As not all supramolecular structures can be readily purified, many have been inaccessible to any method.

An alternative method, called focused ion beam (FIB) milling, uses sputtering of gallium ions—bombarding the sample with high-energy particles—to erode away layers of a frozen-hydrated cell to make the sample suitable for TEM analysis. This FIB technique, borrowed from materials science, has been proven to avoid the artifacts caused by cryosectioning. But to date, researchers have not been able to apply the FIB technique to examine deeply buried structures.

Jürgen Plitzko, Wolfgang Baumeister and co-workers from the Max Planck Institute of Biochemistry in Martinsried, Germany, now describe an improved FIB approach that allows them to target specific, deep intracellular structures for cryoET analysis. Their breakthrough was the development of a milling technique to blast away material both above and below the target region in an ice-embedded cell, to leave behind a thin lamella (<500 nanometers) that is supported by ice on both sides. The procedure can be carried out on a standard TEM sample grid, so transferring the sample to the cryoholder and cryoET analysis is straightforward. The method should be applicable to any cell type that can be grown on or transferred to a TEM grid.

The Plitzko-Baumeister team demonstrated the approach by imaging cytoplasmic regions of *Dictyostelium discoideum* cells and in particular the nuclear pore complex. Though the dataset for this proof-of-principle study was fairly limited, the *in situ* nuclear pore complex structure was similar to that of previous high-quality data obtained from isolated nuclei, and they observed complexes in likely different states of cargo transport.

The FIB technique could be combined with scanning electron microscopy or cryofluorescence microscopy to select structures of interest and guide the milling process. The approach appears poised to open new windows into the mysterious interior of the cell.

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RESEARCH PAPERS

Rigort, A. *et al.* Focused ion beam micromachining of eukaryotic cells for cryoelectron tomography. *Proc. Natl. Acad. Sci. USA* **109**, 4449–4454 (2012).