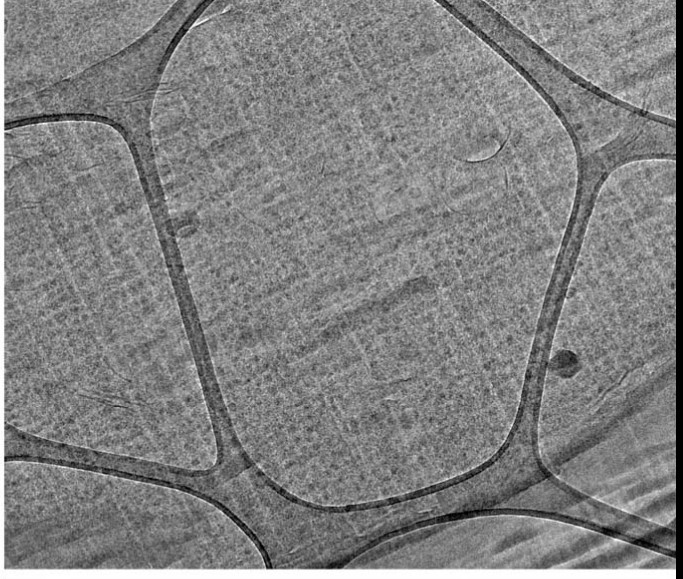
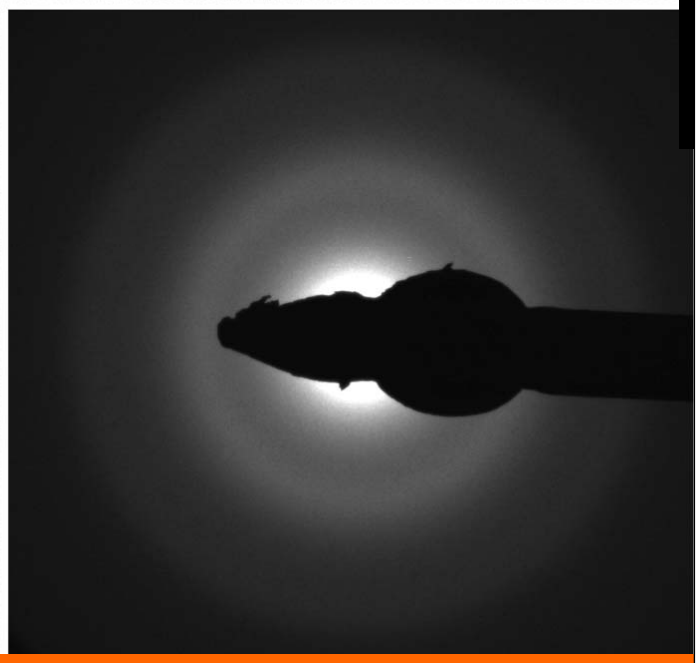




LSN-Application Note

Leica EM HPM100
High Pressure Freezing

CEMOVIS of yeast

Courtesy of Dr. Jonathan O'Driscoll, Dr. Daniel Kofi Clare and Prof. Helen Saibil, prepared at the Department of Cryostallography, Birkbeck, University of London

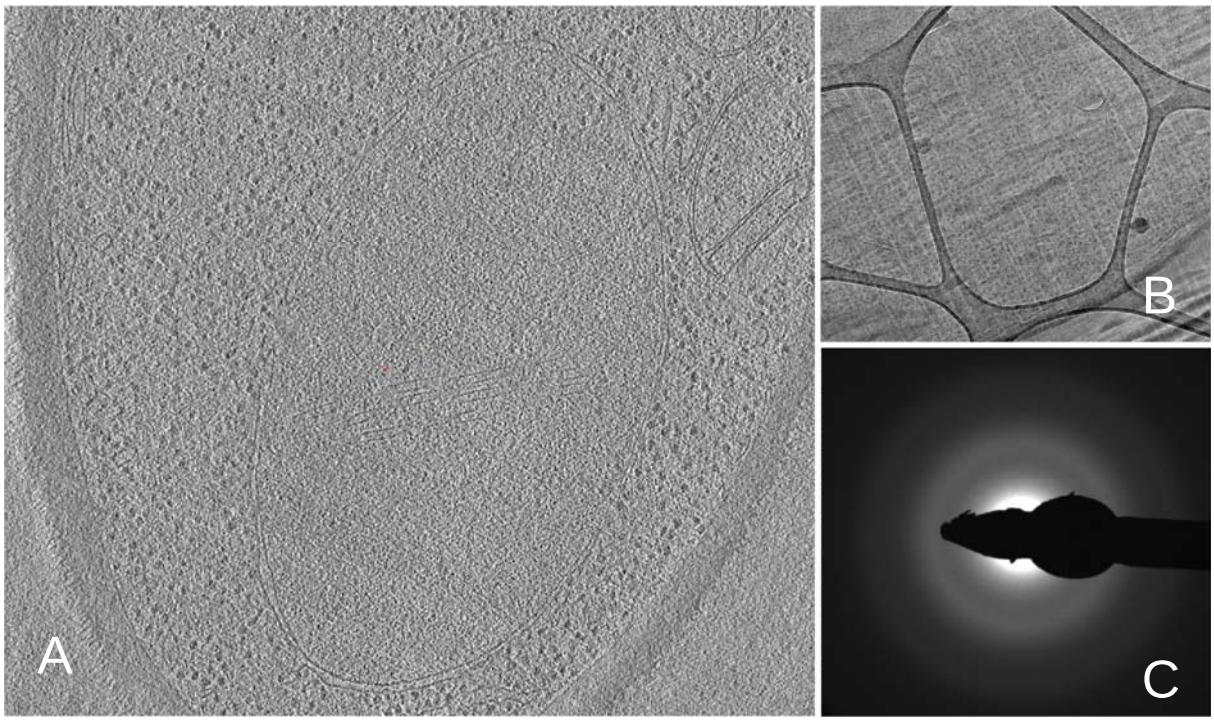
Living up to Life

Leica MICROSYSTEMS

Leica EM HPM100

Protocol:
The sections are of yeast frozen with a Leica HPM100 in the copper tube system, the cell paste was mixed with a pH 6.5 MES/dextran buffer so that a final MES concentration of 50mM and a dextran concentration of 20% was achieved.
The samples were sectioned on a Leica EM UC7/ EM FC7 with Micromanipulator at -140°C and the section thickness was set to 50nm. The sections were attached to Agar lacey grids.

The sections were imaged using a Tecnai Polara 300KeV (FEI, The Netherlands) microscope fitted with a 4K Gatan CCD camera. The magnification for the sections was 23K, with a defocus of -6um for the tomogram and -8um for the projection image, and the diffraction was done with a camera length of 930mm. The image in panel A is an average of the central 10 slices of a reconstruction done with the IMOD package (Kremer et. al., 1996)image processing software from a tomogram collected using the FEI software.



A – optical slice from a tomographic reconstruction
B – micrograph of a vitrified yeast cell
C – diffraction pattern image

Courtesy of Dr. Jonathan O'Driscoll, Dr. Daniel Kofi Clare and Prof. Helen Saibil, prepared at the Department of Crystallography, Birkbeck, University of London