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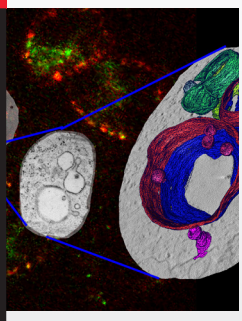
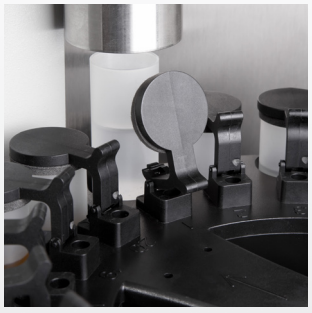
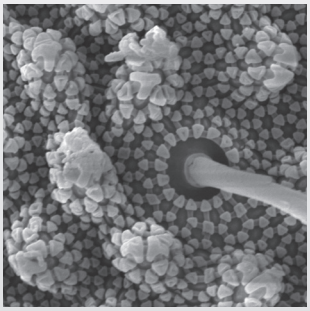

Material Research




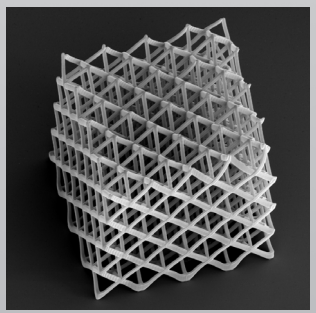


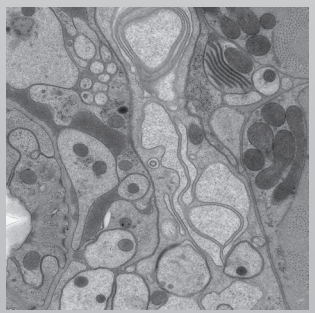

Life Science Research

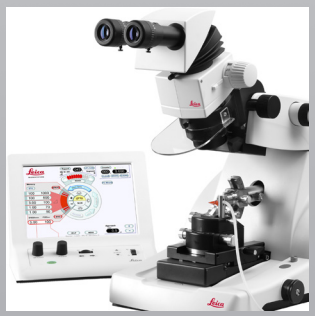
Application Note

Targeting of peroxisomal matrix proteins in the diatom Phaeodactylum tricornutum

related instrument Leica EM PACT2 / EM AFS2

Medical Research



Industrial Manufacturing


Natural Resources



Targeting of peroxisomal matrix proteins in the diatom *Phaeodactylum tricornutum*

COURTESY

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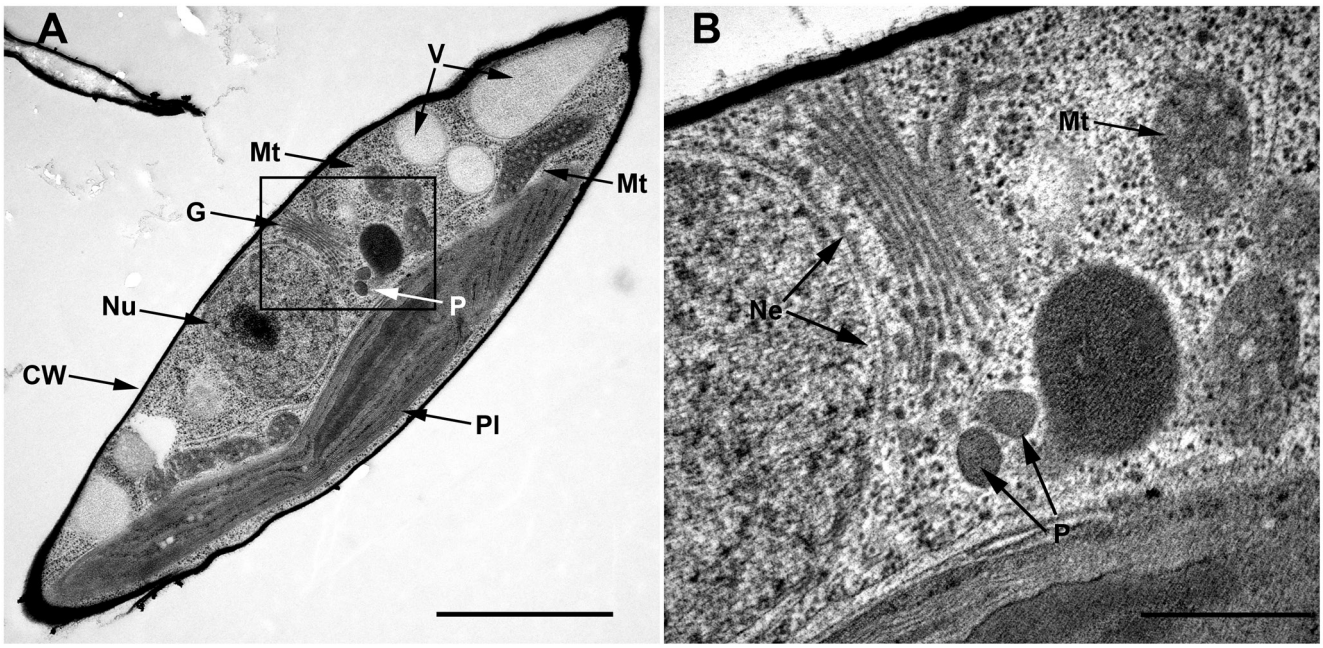
The website of the EM facility is:
http://www.synmikro.com/de/startseite/core-facilities/elektronenmikroskopie.html

SAMPLE PREPARATION:

P. tricornutum cells expressing different types of GFP fusion proteins were harvested via centrifugation at 1,500xg and cryoimmobilized by high-pressure freezing on gold carriers (Leica EM PACT2, Leica Microsystems GmbH, Vienna, Austria). Subsequently, the cells were freeze-substituted with acetone in combination with 2% OsO4, 0.25% uranyl acetate and 5% H2O. Freeze substitution was carried out in the automated Leica EM AFS2 unit (Leica Microsystems GmbH, Vienna, Austria) at -90°C for 4 h, -60°C for 8 h, -30°C for 8 h and then held at 0°C for at least 3 h. The heating time between each step was 1 h. After washing the samples in ice-cold acetone, they were gradually infiltrated in Epon at room temperature, followed by polymerization at 60°C for three days. Ultrathin sections of embedded samples were collected on uncoated nickel grids (400 square mesh). Transmission electron micrographs were either taken on a JEOL 2100 TEM operated at 80 kV in combination with a fast-scan 2K x 2K CCD camera F214 (TVIPS, Gauting, Germany) or on a Zeiss CEM 902 operated at 80 kV equipped with a wide-angle Dual Speed 2K CCD camera (TRS, Moorenweis, Germany).

For more details see:
A single peroxisomal targeting signal mediates matrix protein import in diatoms. Gonzalez NH, Felsner G, Schramm FD, Klingl A, Maier UG, Bolte K, (2011) PLoS ONE 6(9): e25316. doi:10.1371/journal.pone



LEGEND FOR IMAGES:

(A) Ultrathin section of *P. tricornutum* expressing Pex10-GFP in Epon without antibody labeling. The boxed area is shown in (B) at higher magnification and illustrates two peroxisomes in proximity to the nucleus, the golgi and the plastid.

CW, cell wall; G, golgi apparatus; Mt, mitochondrium; Ne, nuclear envelope; Nu, nucleus; P, peroxisome; Pl, plastid; V, vacuole. Scale bars represent 2 µm (A), 500 nm (B).

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RELATED PRODUCTS

Leica EM PACT2

Leica EM AFS2

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