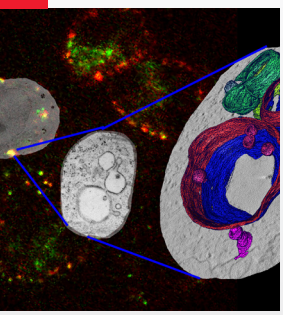
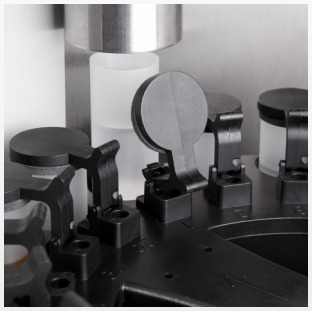
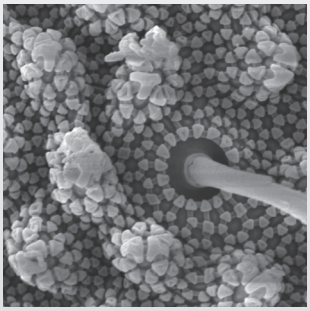
From Eye to Insight

Leica MICROSYSTEMS



Material Research





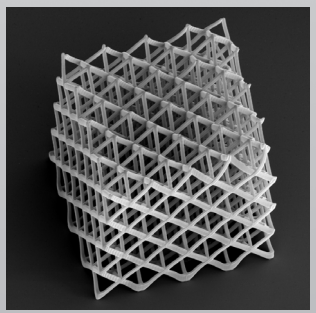
Life Science Research

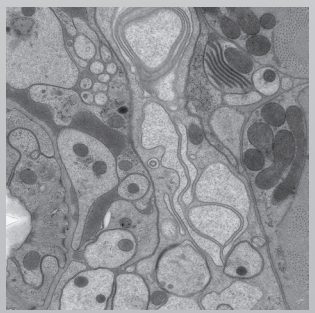
APPLICATION NOTE

Plunge Freezing of Microtubules

related instruments: EM GP2

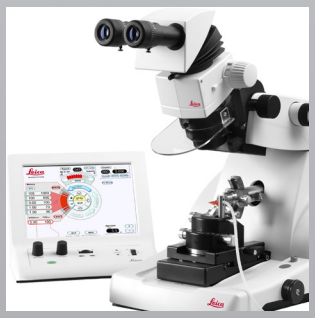
Medical Research





Industrial Manufacturing

Natural Resources



Plunge Freezing of Microtubules

Filter paper blotting/plunge freezing is the single most important specimen preparation technique for cryo-transmission electron microscopy in structural biology, allowing to freeze thin layers of specimen in their natural, hydrated environment without the formation of ice crystals (vitrification). Besides achieving high freezing rates allowing vitrification, it is essential to maintain the specimen under controlled conditions before freezing, to take measures avoiding surface contamination of the frozen specimen by condensing humidity, and to yield reproducible results from one grid to the next.

For freezing microtubules the EM GP2's environmental chamber was set to 25 °C at 95% relative humidity, with the window heater and the nitrogen gas flow in the cryogen Dewar set to 100%. Ethane (Alphagaz N45, 99.995% purity) was condensed using the EM GP2's own liquefier and kept at -182 °C for freezing.

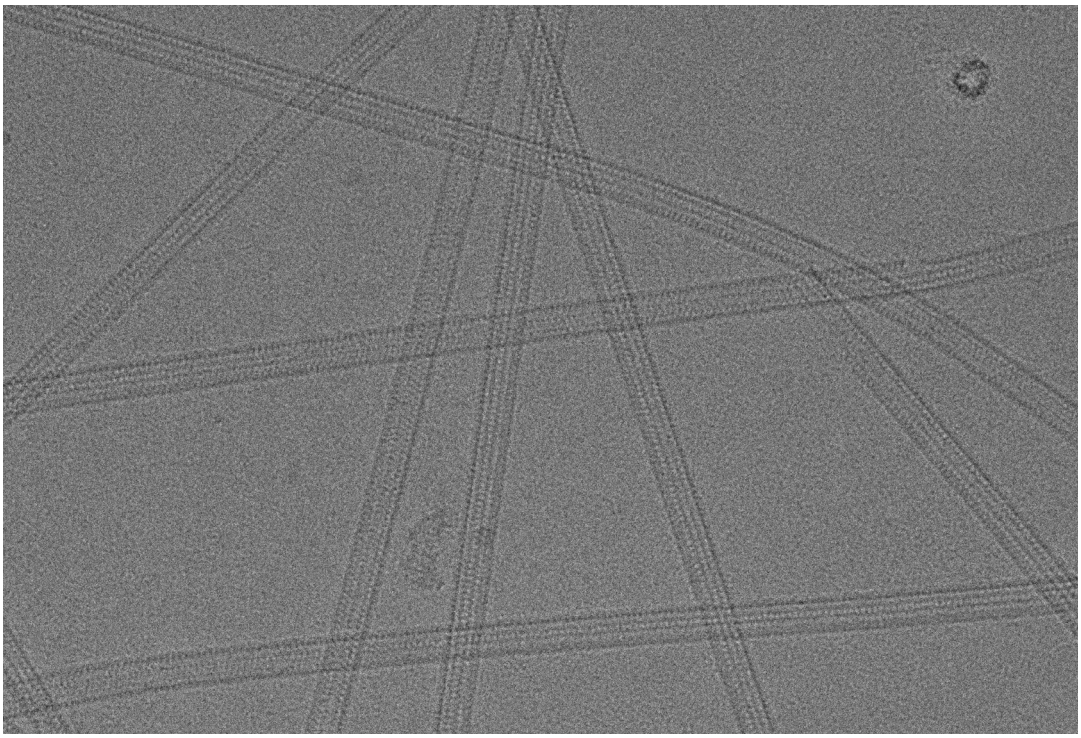
400 mesh Cu grids with Quantifoil R2/2 perforated carbon films (2 µm holes) were glow discharged for 45s and used within 15 minutes of hydrophilisation.

4 µl of a microtubule solution in GPEM buffer (1 mM GTP, 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$) diluted to 0.4 mg/ml tubulin concentration and stabilised with 10 µM taxol were applied onto the carbon side of the Quantifoil grid with a microliter pipette and the sample was adsorbed for 30 seconds. The blotting paper was approached to the grid using the EM GP2's unique blotting sensor with an additional movement of +1.75 mm after contact of the blotting paper with the aqueous solution. The grid was blotted for 1.3 seconds from the carbon film side and plunged without further wait time into the secondary cryogen.

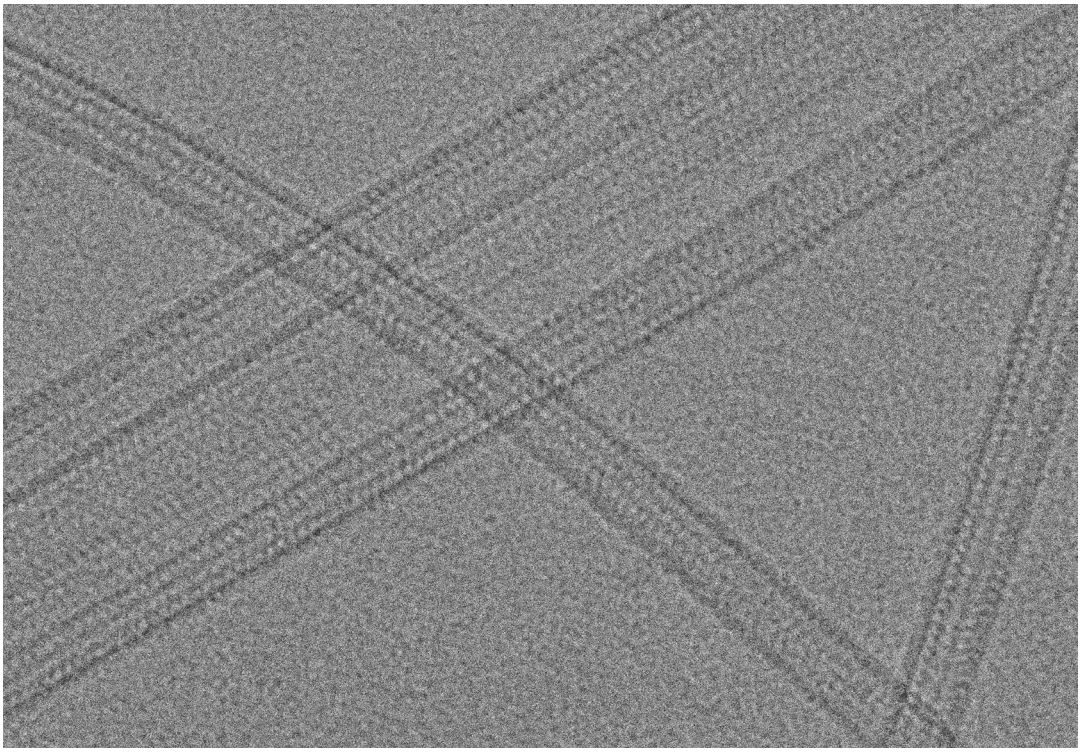
The sample was transferred for storage to a cryo-grid box in liquid nitrogen until samples could be visualised on a FEI Tecnai Polara at 300 kV using a Gatan K2 Summit direct eletron detector in counting mode at a dose rate of 10 e/s/pixel, total dose of 40 e/$Å^2$ and a defocus of -4 to -6 µm.

Micrographs A and B show images of microtubules acquired at 20000x (A) and 39000x (B) nominal magnification in the holes of the perforated carbon film. The ice is free of exogenous contamination, the protofilaments and the individual tubulin subunits are clearly visible along the length of the microtubules.

Courtesy of Dr. Guenter Resch, Nexperion e.U. - Solutions for Electron Microscopy; Dr. Thomas Heuser and Marlene Brandstetter, Vienna Biocenter Core Facilities GmbH



(A) 20000x nominal magnification



(B) 39000x nominal magnification

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EM GP2

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