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IT-10-P-1698 The physical properties of plastic support films for 3-D transmission electron microscopy.

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In the last 10 years, the acquisition of large volumes of biological specimens at high resolution has regained importance, especially in the field of neurobiology. The analysis of large volumes has become essential to better understand cell-cell relationship and the interaction of subcellular organelles.
Though more modern and automated processes like serial block face scanning electron microscopy [1] and focused ion beam scanning electron microscopy [2] ease the process of gaining large volumes, traditional serial section has come to a revival [3].
Serial sectioning maintains its ability to image almost an unlimited 3D volume, up to mm2 at high resolution (X=Y=~1nm; Z=50-70nm). Further serial thick section TEM tomography can improve the Z resolution to about 2nm. The only limit is the skill and persistence of the operator.
For serial section image acquisition and for serial TEM tomography the stability of the plastic support film of the TEM grids, especially large slot grids, is crucial. The following quality criteria are required:
- Flatness, the films should not bend during pick-up of the sections.
- Resilient and strong, the films have to support mechanical tensions.
- Beam resistance, the films should not drift and disrupt in the electron beam.
- Temporal stability, these qualities should be maintained for a long time to allow storage of prepared grids.
To improve the stability of the section on the support film it is important to review the properties of the different polymers commonly used and to find the formulation, which best matches the quality criteria required.
We prepared support films from 6 different polymers and analysed their behaviour during pick-up and TEM imaging. Plastic films were casted on microscope slides and two films of equal thickness were separated. One was used as a support film on a TEM grid and the other was mounted on a slide for AFM analysis. In the AFM the thickness, stiffness and adhesion force was measured. In the TEM, drift measurements were done by following gold particles at high magnification using time lapse series acquisition. Thickness was also measured by TEM tomography.
In conclusion, two polymers emerged that fulfil the requested criteria for 3D investigations by serial sectioning.

1 - Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. Denk W et al.; PLoS Biol. 2004 Nov;2(11):e329
2 - Serial section scanning electron microscopy of adult brain tissue using focused ion beam milling. Knott G et al.; J Neurosci. 2008 Mar 19;28(12):2959-64
3 - Array Tomography: A New Tool for Imaging the Molecular Architecture and Ultrastructure of Neural Circuits. Micheva KD et al; Neuron. 2007 Jul 5;55(1):25-36