Mitochondrial Protrusions in Neuronal Cells

Introduction

The production of adenosine triphosphate (ATP), by mitochondria within eukaryotic cells, powers most of the biochemical reactions which keep the cell healthy and able to perform its functions. Mitochondria are subcellular organelles which uptake and secrete various molecules, in small packets called vesicles, as a mechanism of quality control. Extremely fine cross-sectioning of fixed cells allows for high resolution imaging techniques to aid in the evaluation of mitochondrial structure and function. For example, Yao and colleagues were able to identify newly forming mitochondrial vesicles in neurons via electron microscopy imaging of 30 µm thick brain tissue sections. To generate this large dataset, they used the RMC Boeckeler PowerTome PCZ (PTPCZ) ultramicrotome in combination with the ATUM module to collect hundreds of serial cross-sections on tape for later imaging and 3D reconstruction of entire mitochondria. Because these organelles and sections are so small, it takes considerable resources, time, and care to collect and analyze the volume of images necessary to make scientifically founded conclusions. However, as we describe here, use of the ATUM with PT PCZ can automate this process (for unattended collection) as well as vastly improve the reliability of tissue sectioning and section collection in preparation for light, electron, and other microscopic evaluations.

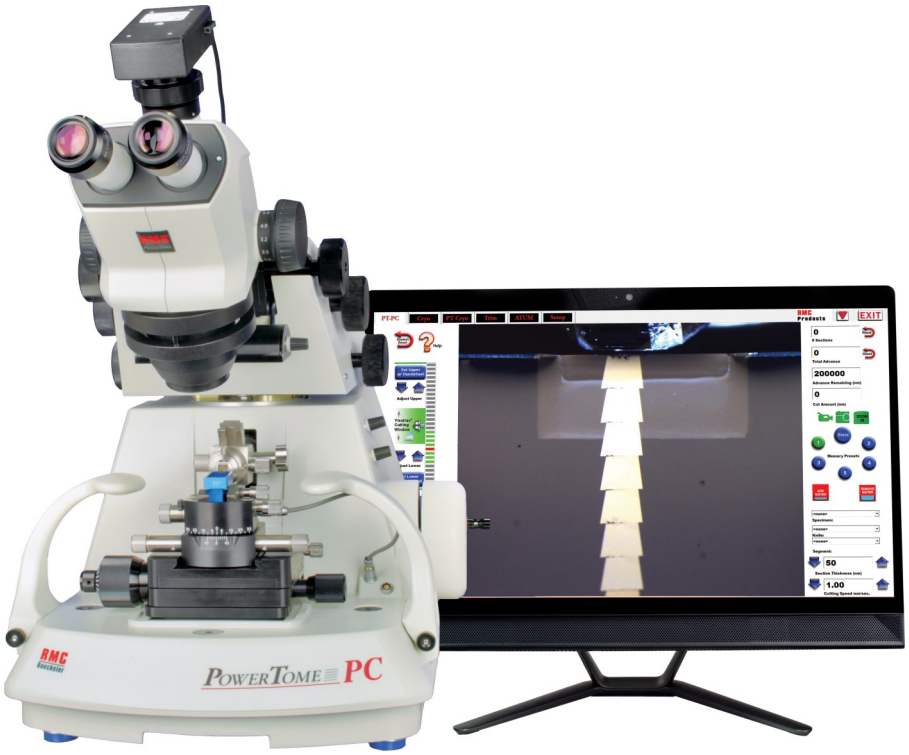

Figure 1.
The RMC Boeckeler PowerTome PCZ ultramicrotome.

Instrumentation

The RMC Boeckeler PT PCZ and an ATUM module was used to serial section mouse cortical samples. The PT PCZ ultramicrotome is equipped with a touchscreen PC and a trinocular microscope with high definition camera. The PowerTome can be controlled and programmed by the touchscreen and/or controller. A camera is useful to visualize and optimize the sectioning of the sample. This is also advantageous in allowing several people to view the process live and simultaneously. The camera can also capture images and videos for use in training, reporting, and documentation. Furthermore, the built-in measuring tool enables measurement of sample block face and embedded sample dimensions by using the image displayed on the touchscreen.

The ATUM module seamlessly attaches to the PT PCZ ultramicrotome and automatically collects the sections as they are cut onto a conveyor belt-like tape and reel system. Many microtomists and microscopists have published their results of using the ATUM for automated, reliable collection of serial sections. The PT PCZ and ATUM are suitable even for large block face sample preparations (e.g. 3x3 mm) and ultrathin sectioning (e.g. 30nm thick). Thus, the ATUM unit ultimately increases the rate and quality of data acquisition.

Procedure

Mouse cerebral cortex was fixed, stained, and embedded in a 7 mm wide flat mold and sectioned using a diamond knife at ≈ 29µm section thickness from a sample block face trimmed to focus on the somatosensory cortex, with dimensions of ≈ 1x1mm square. A total of 1,850 serial sections were collected. Sections were automatically picked up by the ATUM conveyor belt onto Kapton tape. The tape was then manually cut into shorter lengths and mounted on silicon wafers, photographed for mapping, and prepared for automated EM imaging. These wafers are amenable to repeated rounds of various imaging (e.g. multiple resolutions). Sections were imaged with a scanning electron microscope (SEM) using backscattered electron detection (9–10 keV), which had sufficient resolution and contrast to detect individual subcelluar organelles, including vesicles. A reduced osmium tetroxide-thiocarbohydrazide (TCH)-osmium (ROTO) was used as stain.

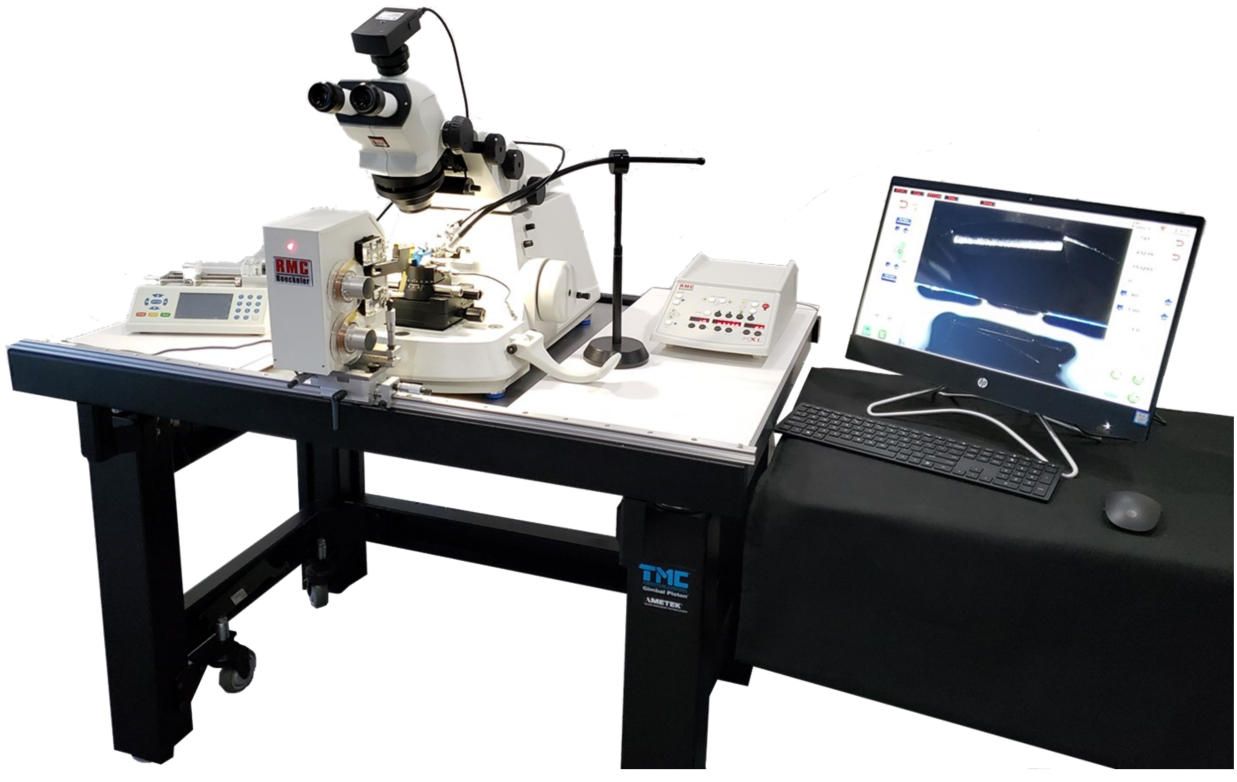

Figure 2.
The RMC Boeckeler ATUM unit in operation with the PT PCZ ultramicrotome.

Results

Application of the RMC Boeckeler PT PCZ ultramicrotome in combination with the ATUM module provides clear advantages in the efficient, reliable, and adaptable collection of serial sections. Yao et al. were able to section a high enough volume to reconstruct a high spatial resolution 3D sample volume and gain statistical insight into the size, shape, and frequency of occurrence of small, sparse subcellular organelles (mitochondria and associated protrusions and vesicles) via SEM (Fig. 3). The modern age of computing and nanotechnology manufacturing promises expanded research capabilities, in this case, the use of serial sectioning and 3D reconstruction, as a powerful approach in research and medical evaluation. The PT PCZ and ATUM represent a prime example of this technological cutting edge for sample preparation instruments.

Acknowledgement

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Yao et. al, 2020. iScience/CellPress Open Access.

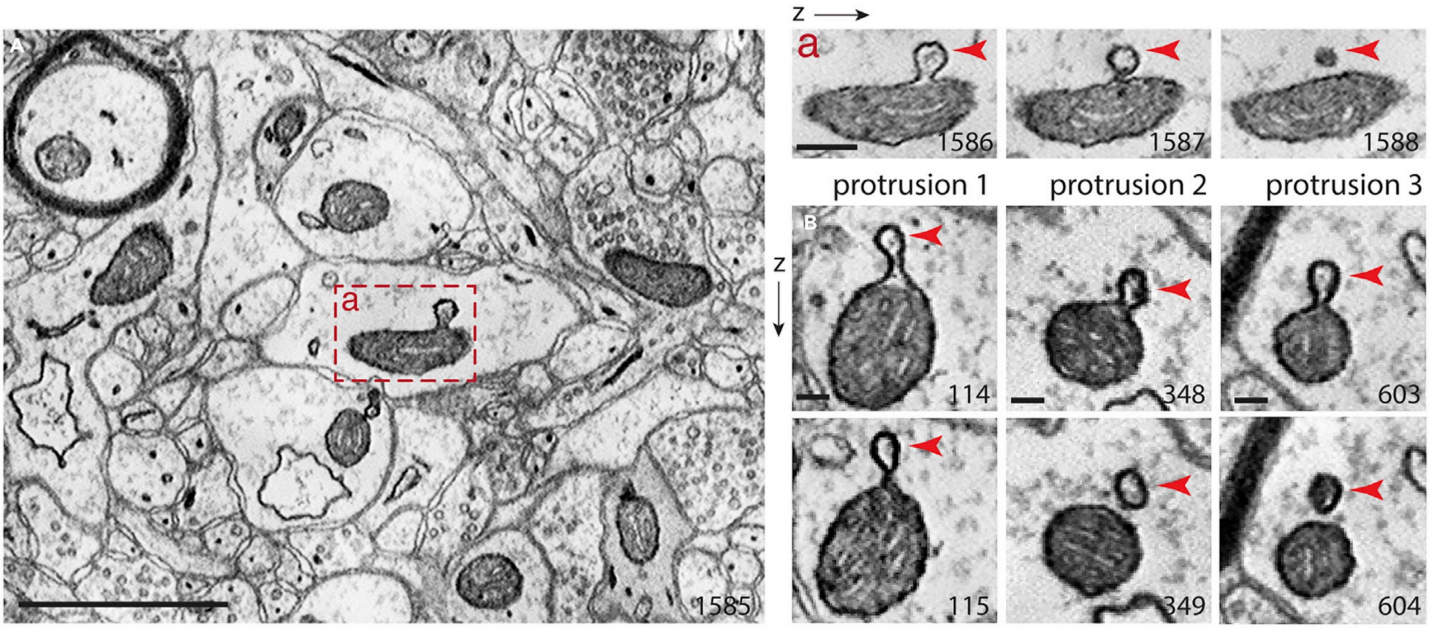

Figure 3. ATUM-SEM Analysis of Mitochondrial Protrusions in Neurons of the Mouse Cortex

(A) A representative micrograph showing mitochondria in axonal or dendritic compartments of neurons. Box-a shows a mitochondrion with a protrusion (arrowheads); note that the tip but not neck of this protrusion stays visible in subsequent micrographs in the z direction (arrowheads). The z resolution of the ATUM-SEM for the cortex is 30 nm. The number on the micrograph indicates its z position in the SEM image stack. (B) Micrographs showing three different protrusions from the same mitochondrion, as shown in Aa. Reproduced from Yao et. al, 2020. Published by iScience/CellPress Open Access.

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