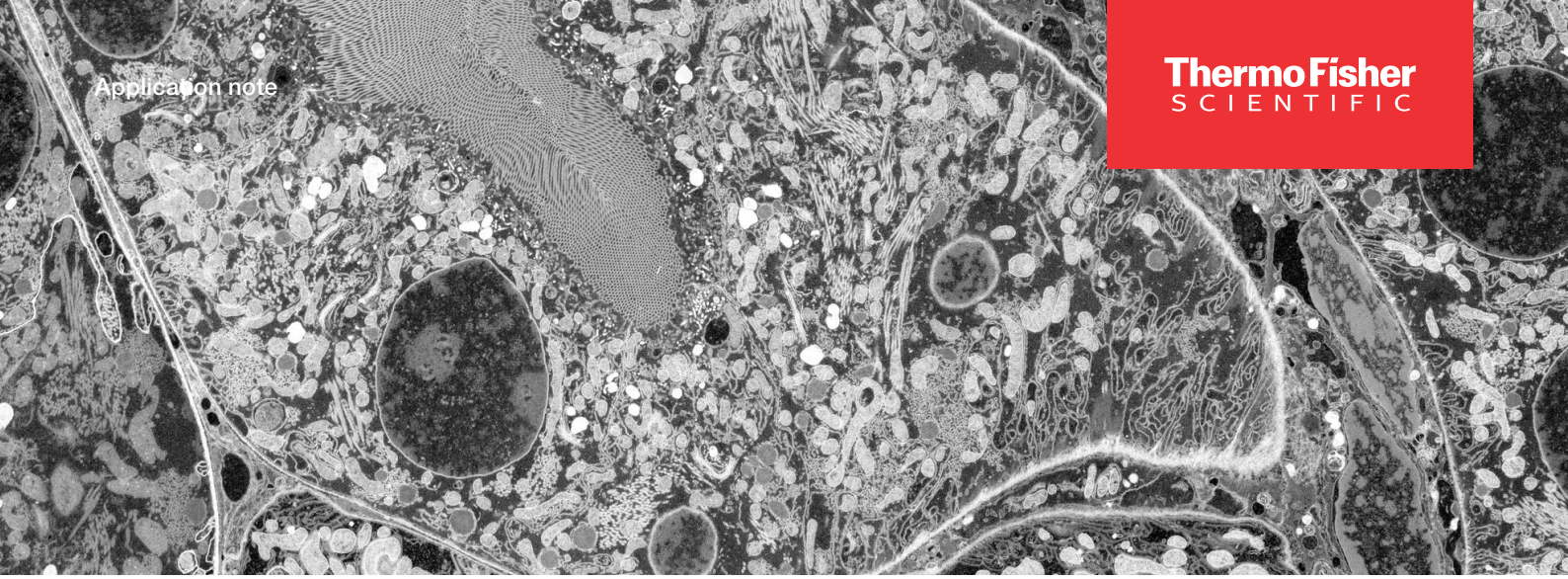

Application note

Room-temperature electron microscopy

Bridging morphology and function in biological research

Room temperature electron microscopy (RT-EM) has long been a cornerstone of biological imaging, enabling the visualization of cells, tissues, and organelles in their natural state under standard laboratory conditions.

The availability of established preparation methods, and its compatibility with diverse model systems, make RT-EM ideally suited for the connection of structural and functional insights. While recent advances in cryogenic electron microscopy (cryo-EM) have driven a number of critical investigations at near-atomic resolution, these techniques are constrained by specimen thickness. (I.e., up to ~200 µm with high-pressure freezing.) The use of chemical fixation in RT-EM, together with established protocols, allows these methods to examine larger model systems.

The complexity of applied biological research

In applied research fields such as organismal morphology, anatomy, pathology, pharmaceutical development, neurobiology, and cell biology, ultra-high resolution is not always the primary requirement. Instead, these disciplines often demand complex experimental designs (such as behavioral assays, longitudinal studies, and genetic modifications) that benefit from flexible and scalable imaging approaches. Broad morphological assessments, at both intracellular and extracellular levels, are frequently more relevant than atomic-level detail. In such contexts, the highly specialized conditions required for cryo-EM may not align with every experimental need. For example, comparative studies of disease models often use animal systems like mice, rats, flies, or small worms. Pathological phenotypes may develop at the ultrastructural level and affect general tissue morphology, reflecting a complex interplay of disease mechanisms (Figure 1).

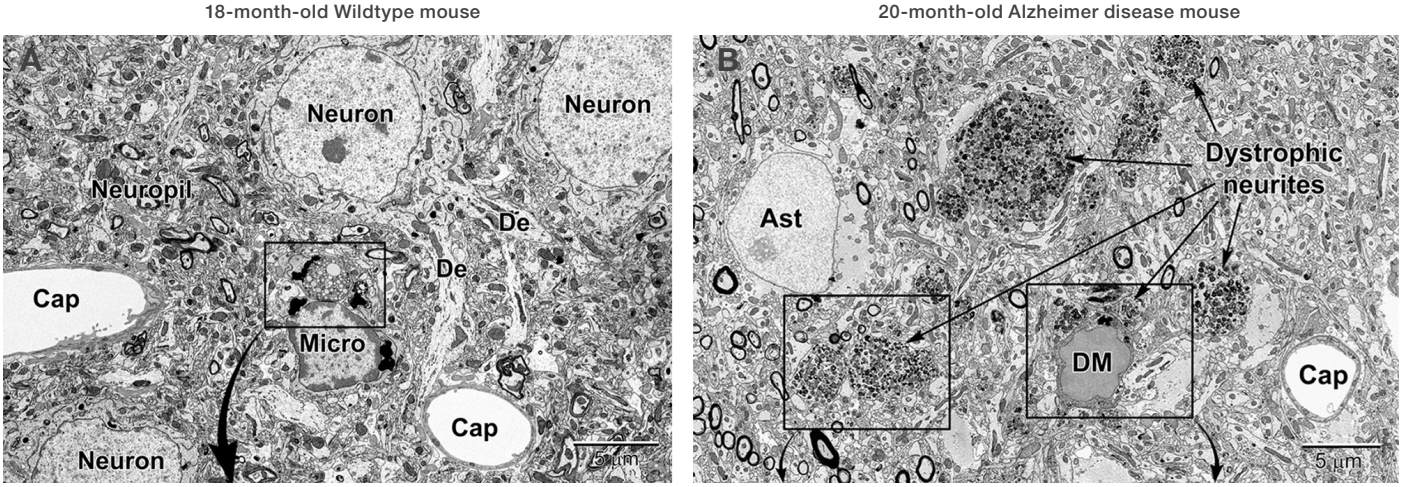

Figure 1. Comparison of ultrastructural pathology in wild-type mice and those afflicted by Alzheimer's disease. Figure reproduced from Nahirney and Tremblay under CC BY 4.0. (doi: 10.3389/fcell.2021.629503)

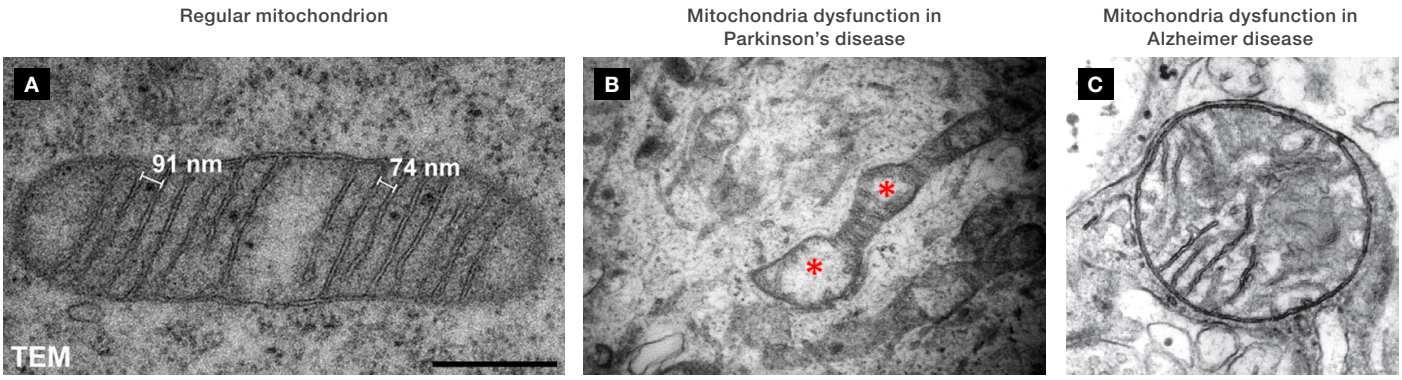

Figure 2. Mitochondrial dysfunction investigated with room temperature electron microscopy. A) A regular mitochondrion. B) Ultrastructural mitochondrial abnormalities in dopaminergic neurons observed in Parkinson's disease models. C) Disruption of mitochondrial cristae. Figure adapted from Stephan et al. (doi: 10.1038/s41598-019-48838-2), Vetchinova et al. (doi: 10.3390/cimb45100529) and Baloyannis (doi: 10.5772/intechopen.84881) under CC BY 4.0 and CC BY 3.0.

Benefits of room-temperature EM

Room-temperature electron microscopy provides an alternative imaging approach to cryo-EM that operates under standard laboratory conditions. It has a long-standing history in biological research, having been widely used for decades to study cellular and tissue ultrastructure. RT-EM uses well-established chemical fixation methods and can be integrated into a variety of experimental pipelines. Importantly, RT-EM can be performed with both transmission (TEM) and scanning electron microscopy (SEM), enabling a range of high-resolution analyses, from thin sections to 3D surface and volume investigations. RT-EM is compatible with workflows that combine structural and functional insights, and particularly multimodal setups, which are often necessary in diverse research environments. Figure 2 shows an example study leveraging RT-EM; mitochondrial dysfunction (as observed in diseases such as Parkinson's and Alzheimer's) was investigated at the ultrastructural level, revealing cellular and neuronal alterations.

RT-EM bridges the gap between functional techniques (such as light microscopy, genetic analysis, and behavioral studies) and high-resolution approaches like cryo-EM. It enables researchers to correlate functional and morphological data across spatial and resolution scales, contributing to a comprehensive understanding of biological systems. Such versatile toolkits provide both high precision and flexibility, as needed, meeting the diverse demands of modern life sciences.

Streamlined RT-EM workflows from Thermo Fisher Scientific

Room temperature EM workflows are straightforward and adaptable to a wide range of research settings. They can be applied to many sample types, and integrate well with existing experimental approaches.

- Applicable to large and complex samples, including whole cells, tissues, organoids, and animals
- Has established methods and protocols for sample preparation (To learn more, see: Electron Microscopy: Methods and Protocols (ISBN: 9781627037754) and Introduction to Electron Microscopy for Biologists (ISBN: 9780123743206))
- Does not typically require freezing or cryogenic handling, but can be combined with hybrid methods such as cryo-sectioning (i.e., Tokuyasu sectioning) or freeze substitution
- Allows visualization of structural processes such as mitochondrial fission and fusion, vesicle trafficking, endosomal maturation, and synaptic remodeling
- Suitable for comparing wild-type, genetically modified, and drug-treated animal models through the analysis of morphological changes
- Compatible with complementary techniques such as light microscopy, electrophysiology, behavioral assays, correlative workflows, and immuno-gold labeling for targeted molecular identification

By combining accessibility with versatility, RT-EM serves as a valuable tool for the investigation of biological systems in a broader experimental context.

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