Application note

Image data courtesy of Dr. Benjamin Engel, Dept. of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.

Generating surfaces and measuring 3D organelle morphology with Amira Software

Organelles carry out the fundamental processes that keep cells (and hence tissues, organs and organisms) alive and functioning. In recent decades, light microscopy and transmission electron microscopy (TEM) have proven instrumental in elucidating the fundamental structures of organelles. However, these are 2D techniques – and a thorough understanding of organelle structure and interaction within living cells can only be achieved through the acquisition of detailed 3D images. High-resolution 3D imaging of organelles is, therefore, one of the fundamental goals of cell biology.

This application note explores a **2022 study** published in Cells where researchers combined Thermo Scientific™ Amira™ Software with serial block-face scanning electron microscopy (SBF SEM) in order to produce detailed 3D reconstructions of organelles. By directly measuring organelle morphology, the authors were able to investigate the effects of gene inhibition on the function and morphology of mitochondria and the endoplasmic reticulum.[1]

The roles of metabolic organelles

Metabolic organelles such as mitochondria and the endoplasmic reticulum (ER) play vital roles in a huge range of cellular processes.

Mitochondria are well-known as the organelles responsible for the generation of ATP via oxidative phosphorylation. This process is the crucial final step in cellular respiration: ATP is used as the primary energy source for most biochemical and physiological processes such as growth, movement, and homeostasis. Mitochondrial oxidative phosphorylation is therefore vital in order for animals, fungi, and bacteria to extract energy from oxygen and food. This ability has earned mitochondria the title of “the powerhouse of the cell,” but they do, however, have additional functions beyond ATP production.

We now know that mitochondria play important roles in apoptosis (programmed cell death) and mitosis (cell division).[2–4] For example, dynamin-related protein-1 (DRP-1) is associated with the early stages of apoptosis and thus controls mitochondrial fission. Changes in mitochondrial ultrastructures are also associated with changes in the chemical pathways that regulate important biomolecules such as calcium and potassium. This mitochondrial regulation of calcium appears to play a role in apoptosis while also affecting ER calcium levels which, in turn, regulate mitosis.[5]

As a result of their roles in a diverse range of biological processes, both mitochondria and the ER are promising pharmaceutical targets for the treatment of neurodegenerative and viral diseases, as well as cancer.

Understanding organelle form and function

While there is much information on the biochemical profiles of organelles, comparatively little is known about the finer points of their morphology or their interactions with each other at relatively large cellular length scales.

Developing a more complete understanding of organelle function is widely considered to only be possible through high-resolution 3D scanning of organelles within intact cells.

Mitochondria are just one example of how high-resolution 3D imaging can elucidate the relationship between form and function in cellular biology. The inner membrane of mitochondria is pleated into folds known as cristae. While these folds appear ribbon-like in a 2D plane, they are in fact tubular structures that vary in both area and volume. 3D reconstructions of mitochondria have provided biologists with an enhanced understanding of calcium stores, the spatial distribution of metabolites, and the size of cristae.[6] 3D visualization has also provided insights into the structure and function of other subcellular structures such as the ER and microtubules.[7]

Electron microscopy in cell biology

3D imaging of cells has long proven elusive – though recent developments in scanning electron microscopy offer a few promising approaches.

SEM relies on the detection of backscattered electrons produced when a focused beam of primary electrons interacts with a sample. Though SEM itself is effectively a 2D imaging technique, using it in conjunction with ultra-thin sectioning can build up a 3D image by scanning layer-by-layer through a sample.

For example, focused ion beam SEM (FIB SEM) uses the ion beam to precisely ablate the top layer of a sample after it has been imaged by SEM, successively scanning and removing layers. While this results in the destruction of the sample, individual 2D scans can be registered and analyzed to produce 3D reconstructions with unprecedented resolution.

SBF SEM is a related technique that replaces the FIB with an in-chamber ultra-microtome/diamond knife to slice off thin layers between successive SEM scans. While FIB SEM typically offers greater z-axis resolution than SBF SEM (and therefore provides finer details of structures like cristae and nanotunnels), SBF SEM generally enables faster imaging of larger sample volumes.

Reconstructing and analyzing 3D organelles with Amira Software

Producing 3D images of organelles relies on software capable of segmenting and reconstructing 3D structures from “stacks” of 2D images. While numerous software solutions have been developed for these tasks, Amira Software has the unique advantage of combined segmentation and 3D reconstruction, enabling researchers to accelerate experimental workflows.

Amira Software is highly versatile and customizable: it offers an animation creation tool, the ability to assign different colors or “materials” to different organelles, compatibility with a wide range of import and export files, and the creation of workflows with either manual or semi-automated segmentation.

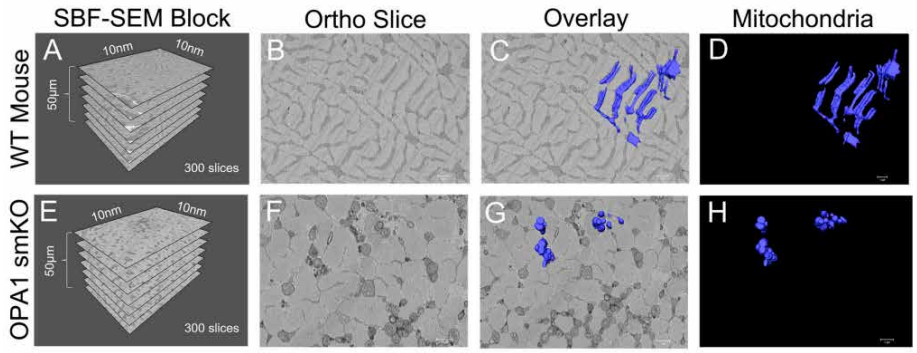

Figure 1. (A) The dimensions of the captured tissue in wild type mouse and (E) OPA1 smKO. (B,F) Example ortho slices for each. (C, G) The overlay of the 3D surface rendering of mitochondria for each. (D,H) The 3D surface rendering of mitochondria alone[1].

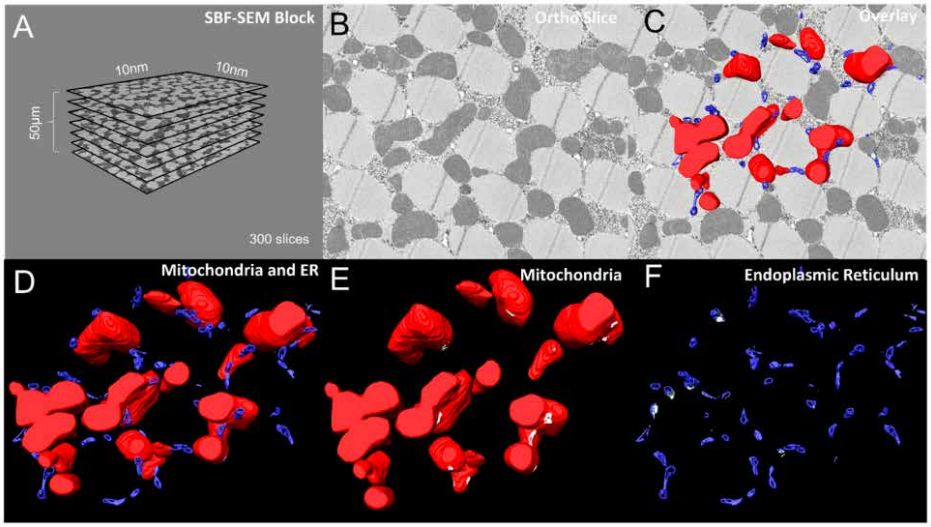

Figure 2. (A) Dimensions and quantities of Drosphilia-flight-muscle ortho slices used for data acquisition and conversion to 3D models. (B) A single ortho slice, (C) with overlaid 3D reconstructions of mitochondria (red) and ER (blue). (D) 3D reconstruction of mitochondria and ER. (E, F) Visualizations of mitochondria only (red) and ER only (blue) respectively, with MERCs shown in white[1].

Investigating the impact of specific genes on organelle morphology

The knockdown effects of two specific genes (optic atrophy-1 (OPA1) and mitofusin-2 (MFN-2)) on structure-function relationships in mitochondria and the ER were investigated in mKO-derived skeletal muscle cells.

The OPA1 gene, which is active in the cristae of mitochondria, plays a number of important roles related to mitochondrial shape and structure, including mitochondrial fusion and apoptosis.[8,9] The gene is also involved in oxidative phosphorylation and in the maintenance of mitochondrial DNA (mtDNA).

The MFN-2 gene is also known to play a critical role in mitochondrial structure. MFN-2 codes for a protein called mitofusin 2, which exists in the outer membrane of mitochondria and

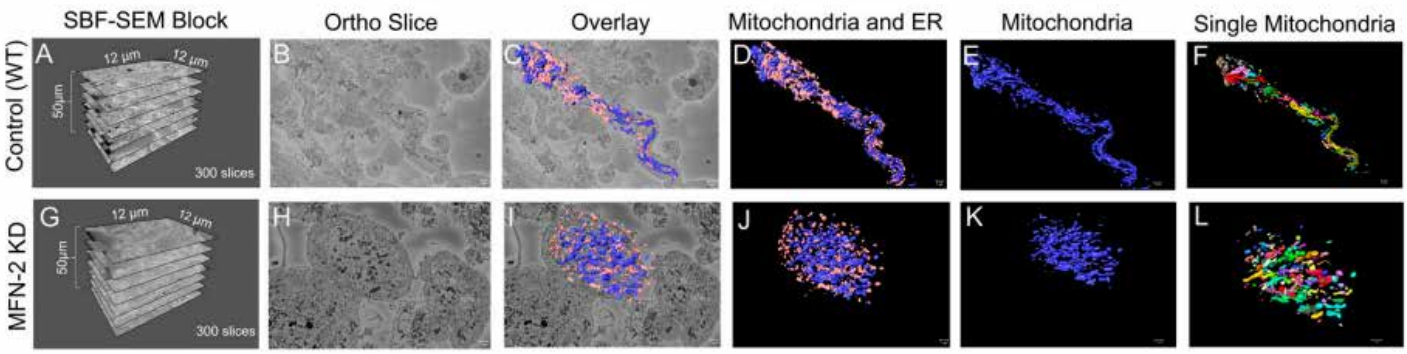

Figure 3. (A–D) The dimensions of the SBF-SEM tissues, an isolated ortho slice, a 3D reconstruction overlay, and an isolated 3D reconstruction of the wild-type myotubes. (E) 3D reconstructions of the wild-type myotubes are also shown, either with mitochondria individually colored or (F) the ER individually colored. (G–J) The dimensions of the SBF-SEM myotubes, an isolated ortho slice, a 3D reconstruction overlay, and an isolated 3D reconstruction of the MFN-2 deficient-myotubes. (K) 3D reconstructions of mitochondria can be shown as a single color or (L) individually colored[1].

determines mitochondrial shape and structure.[10] Mitofusin 2 is known to regulate the shape of mitochondria by contributing to mitochondrial fusion.

SBF SEM was used to image control and knockdown cells while Amira Software subsequently segmented and analyzed the image data. Mitochondria and ER volumes were measured and the contact sites between these organelles were compared before and after the knockdown of each gene of interest.

Results and discussion

Mitochondrial changes in OPA1 knockdown smKO-derived skeletal muscle

As OP1 promotes mitochondrial fusion (thus increasing the volume of mitochondria), it was anticipated that OPA1 knock-down would reduce mitochondrial volume by inhibiting fusion.

Amira Software and SBF SEM were used to produce 3D reconstructions of mitochondria from the gastrocnemius muscle in OPA1 smKO mice. High-quality 3D reconstructions enabled the identification, quantification, and measurement of mitochondria in both samples. Overlaying 3D reconstructions on ortho slices provided a way to visualize the positions of 3D organelles within the context of a sample, while isolated 3D reconstructions revealed finer details.

The animation tool in Amira Software was used to produce videos showing mitochondria from multiple angles. Using these visualization methods, researchers were able to verify their predictions: the mitochondria in OPA1 cells were substantially reduced in both length and volume in comparison to the wild-type controls. The 3D data indicates that OPA1 mutants created fragmented, smaller mitochondria as a result of heightened fission/reduced fusion caused by reduced OPA1 levels.

Identifying mitochondria-ER contact sites (MERCs) Using 3D reconstructions of Drosophila flight muscle

Mitochondria and the ER overlap in function, with both playing a crucial part in progression, metabolism, and apoptosis through the cell cycle. As a result, there is great interest in the sites where the two organelles intersect. Known as mitochondria-ER contact sites, or MERCs, these sites perform many important functions, including facilitation of calcium transfer from the ER to mitochondria as well as roles in bio-signaling.[11] In particular, lipid and calcium signaling that is facilitated by MERCs is essential for mitochondrial fusion and fission.

Amira Software was used to generate 3D reconstructions of both mitochondria and the ER in Drosophila flight muscle cells. Once again, sample ortho slices were overlaid onto 3D reconstructions of mitochondria and ER in order to visualize the positions of these structures within the cell. Additionally, the mitochondria and ER were separately segmented using different “materials” in Amira Software for clear identification and quantification of the MERCs.

Segmentation and reconstruction allowed the relative positions and orientations of mitochondria and the ER within the cell to be clearly visualized. By “graying out” the contact points between the 3D mitochondria and ER models, Amira Software made it easy to view the MERCs themselves – something which is otherwise very difficult to accomplish.

Once again, Amira Software was used to produce animated videos containing multiple MERCs along with comprehensive visualization of mitochondria and the ER. This animation provides a more detailed illustration of the sizes of MERCs throughout the mitochondria.

Investigating the effects of MFN-2 knockdown on organelle morphology

Mitofusin-2 serves as a physical tether between the ER and mitochondria and mediates calcium exchange between them; it is also required for mitochondrial fusion. Knockdown of the MFN-2 gene, therefore, triggers ER stress and mitochondrial dysfunction.

In MFN-2, the role of MERCs is somewhat controversial, though several studies have demonstrated that loss of MFN-2 increases mitochondria-ER coupling.[12,13] MFN-2 becomes important for the effective functioning of cells as MERCs are central to cellular homeostasis.

Amira Software was used to produce high-quality 3D reconstructions of MFN-2-deficient myotubes; the morphology of mitochondria in MFN-2 knockdown myotubes was clearly compared to wild-type controls. Pseudo-coloring was used to provide an alternative means of viewing mitochondria and MERCs.

Amira Software was used to quantify the changes in MFN-2 deficient myotubes in terms of the size, shape, and connectivity of mitochondria and the ER. The volumes and lengths of the MFN-2 mitochondria decreased in comparison to the wild-type controls and exhibited reduced sphericity. These results suggest that fusion abnormalities result in mitochondria that are no longer functional. The decreases in MERC length and volume indicate changes in MERCs, as shown in prior work.

Conclusions

These three studies demonstrate the power of Amira Software in SBF SEM image data processing, producing detailed 3D constructions of organelles. High-quality 3D models created with Amira Software provide clear visualization of organelle distributions and interactions and elucidate the finer points of morphology. This enables researchers to directly observe the effects of genetic changes on the morphology of individual organelles rather than relying on biochemical signals to quantify their behavior.

Amira Software offers unparalleled performance in data visualization for a range of microscopy methods, including SBF SEM, FIB SEM, (μ)CT, and MRI, making it the visualization software of choice for cell biology applications.

References and further reading

1. Garza-Lopez, E et al. *Protocols for Generating Surfaces and Measuring 3D Organelle Morphology Using Amira.* **Cells** 11, 65 (2022)
2. Bratic, A and Larsson, NG. *The role of mitochondria in aging.* **J Clin Invest** 123, 3 (2013)
3. Olichon, A et al. *Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis*. **J Biol Chem** 278 (2003)
4. Otera, H et al. *Drp1-dependent mitochondrial fission via MiD49/51 is essential for apoptotic cristae remodeling.* **J Cell Biol** 212 (2016)
5. Pinton, P et al. *Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis*. **Oncogene** 27 (2008)
6. Wolf, SG et al. *3D visualization of mitochondrial solid-phase calcium stores in whole cells*. **eLife** 6:e29929 (2017)
7. Heinrich, L et al. *Whole-cell organelle segmentation in volume electron microscopy.* **Nature** 599 (2021)
8. Suga, S et al. *An interactive deep learning-based approach reveals mitochondrial cristae topologies.* **bioRxiv** https://www.biorxiv.org/content/10.1101/2021.06.11.448083v1 (2021)
9. *OPA1 gene* https://medlineplus.gov/genetics/gene/opa1/ **MedlinePlus Genetics**
10. *MFN2 gene* https://medlineplus.gov/genetics/gene/mfn2/ **MedlinePlus Genetics**
11. Rowland, AA and Voeltz, GK. *Endoplasmic reticulum–mitochondria contacts: function of the junction.* **Nature Reviews Molecular Cell Biology** 13 (2012)
12. de Brito, OM and Scorrano, L. *Mitofusin 2 tethers endoplasmic reticulum to mitochondria.* **Nature** 456 (2008)
13. Filadi, R et al. *Highlighting the endoplasmic reticulum-mitochondria connection: Focus on Mitofusin 2.* **Pharmacological Research** 128 (2018)

Learn more at **thermofisher.com/amira**

thermo scientific