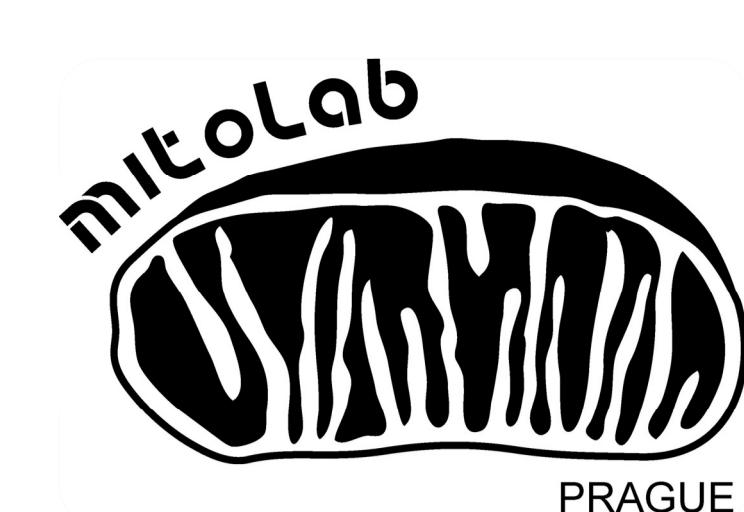




Analysis of mitochondrial ultrastructure and morphology of mitochondrial cristae in relation to the type of mitochondrial dysfunction



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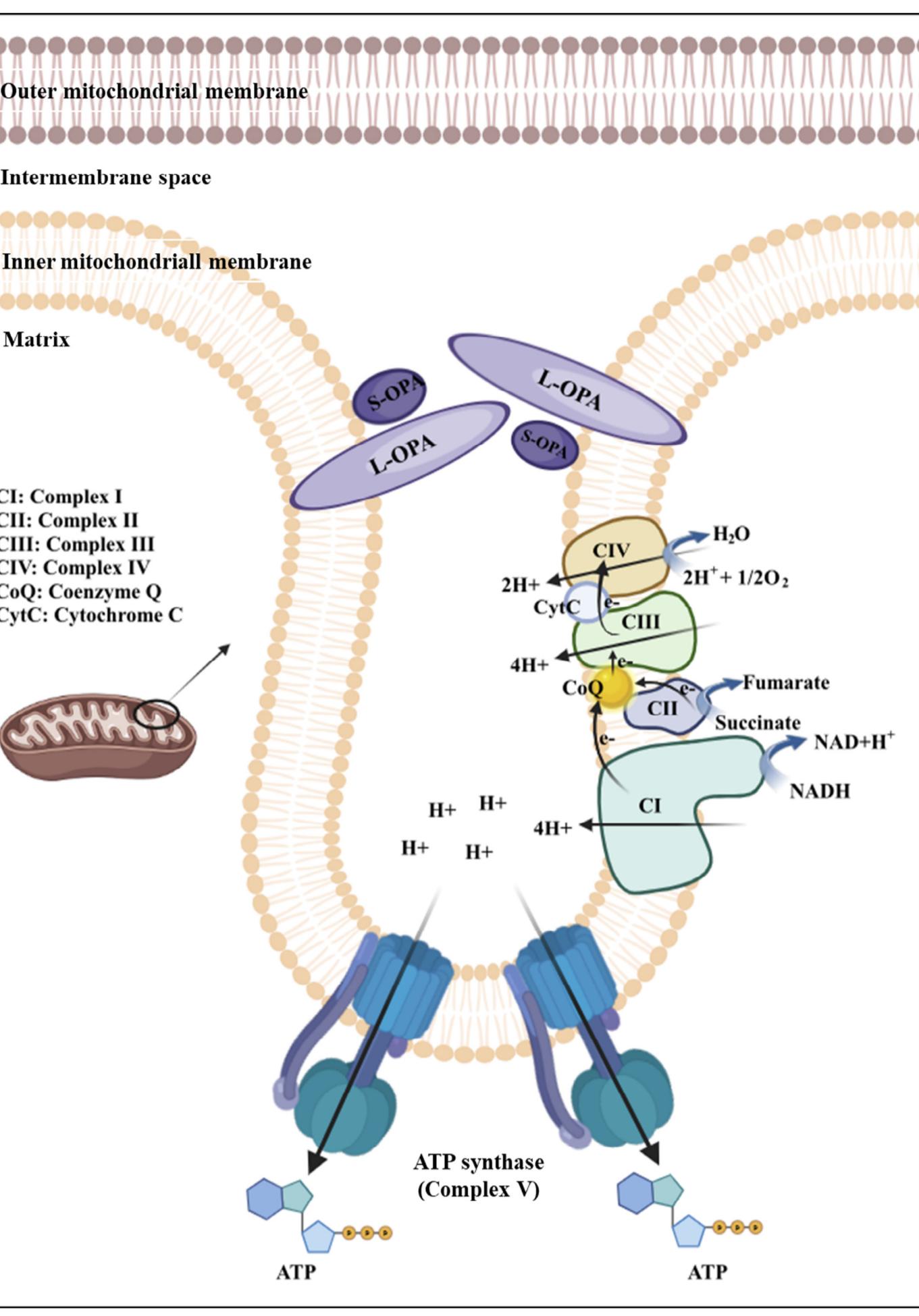
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Introduction: Mitochondria are essential organelles responsible for producing most cellular ATP through oxidative phosphorylation (OXPHOS), making them central to energy metabolism and general cellular homeostasis. In parallel with energy production, they play a key role in apoptosis, calcium signaling, and the regulation of reactive oxygen species (ROS). Due to their high metabolic demands, tissues such as the brain, heart, skeletal muscle, and retina are particularly sensitive to mitochondrial dysfunction. Primary mitochondrial disorders, which occur as a result of mutations in mitochondrial DNA (mtDNA) or nuclear genes encoding OXPHOS components, lead to impaired ATP production and a wide range of clinical manifestations, from neurodegenerative diseases to cardiomyopathies and systemic metabolic syndromes.

The structure of mitochondrial cristae is closely linked to OXPHOS efficiency, and changes in cristae morphology are a characteristic feature of many mitochondrial disorders. In healthy mitochondria, cristae are tightly folded, maximizing surface area for energy production. However, in various mitochondrial diseases, cristae may appear swollen, disorganized, or fragmented, reflecting underlying disturbances in energy metabolism. These structural abnormalities often correlate with disease severity and may vary between tissues depending on their energy requirements. Thus, analysis of cristae morphology provides important information about the functional state of mitochondria and serves as a crucial



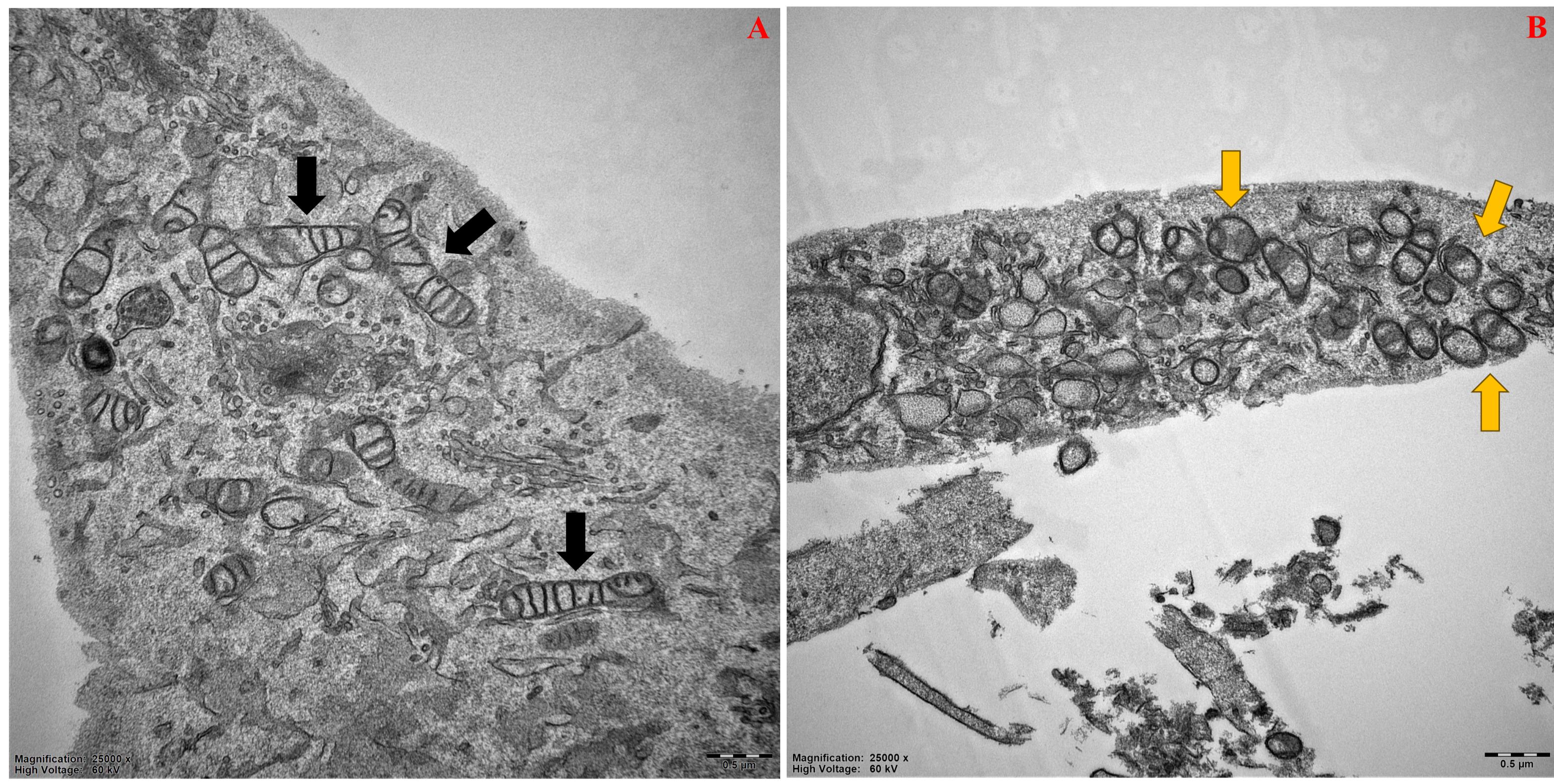
Simplified diagram of mitochondrial cristae lamella and Mitochondrial Oxidative Phosphorylation (OXPHOS) Pathway

This figure illustrates the organization and function of the electron transport chain and ATP synthase in the inner mitochondrial membrane, the site of aerobic energy production.

- Complex I (CI) – NADH:ubiquinone oxidoreductase: Oxidizes NADH → NAD⁺, transfers electrons (e⁻) to CoQ and simultaneously transfers 4H⁺ to the intermembrane space.
- Complex II (CII) – Succinate dehydrogenase: Oxidizes succinate → fumarate, transfers electrons to CoQ without proton translocation.
- Coenzyme Q (CoQ): A lipid-soluble carrier that shuttles electrons from CI and CII to CIII.
- Complex III (CIII) – Ubiquinol-cytochrome c reductase: Transfers electrons to CytC and translocates 4H⁺ across the membrane.
- Cytochrome C (CytC): A mobile electron carrier delivering electrons to CIV.
- Complex IV (CIV) – Cytochrome C oxidase: Reduces O₂ → H₂O, coupled with proton translocation (2H⁺).

The resulting proton gradient (ΔpH) drives ATP synthase, which synthesizes ATP from ADP + Pi, providing the cell with usable chemical energy.

OPA proteins are also shown: L-OPA (long form) and S-OPA (short form), which are crucial for maintaining the structure of mitochondrial cristae and preserving efficient energy production.



Transmission electron microscopy - Jeol 1400 plus (Akishima) (25 000x magnification)

Figure A shows the ultrastructure of mitochondria in cells from a control line of human skin fibroblasts. The black arrows indicate elongated mitochondria with a generous representation of mitochondrial cristae.

Figure B shows the mitochondrial ultrastructure in a cell line of skin fibroblasts from a patient with a mutation in the TMEM70 gene, which is crucial for the proper biogenesis of ATP synthase. Mitochondria are rather round, and the number of cristae is minimal (yellow arrows). Pictures were performed according to Luft (1956), with minor modifications.

Procedure for classification of the general morphology of mitochondria and mitochondrial crystals:

To lower the complexity of the task; we use a two-step approach to classify morphology of cristae by using techniques of deep learning:

STEP 1: Instance segmentation of mitochondria in original EM images

STEP 2: Panoptic segmentation of cristae in segmented images of mitochondria

With the help of **Empanada** by retraining the original MitoNet_v1 model (instance) or using CEM pretrained weights (panoptic) we created two new models:

STEP 1: For instance segmentation of mitochondria using an annotated training set of 3628 patched images (512x512 pixels) created from 189 original images and using 30 training epochs.

Our model, test data - 22 images:

IoU = 0.798

IoU (0.5) = 0.876

Original MitoNet_v1, test data - 22 images:

IoU = 0.596

IoU (0.5) = 0.766

STEP 2: For panoptic segmentation of cristae (giving the possibility to distinguish 12 morphological classes) using an annotated training set of 3716 patched images (512x512 pixels) created from 271 images of segmented mitochondria and using 200 training epochs.

Our model, test data - 28 images:

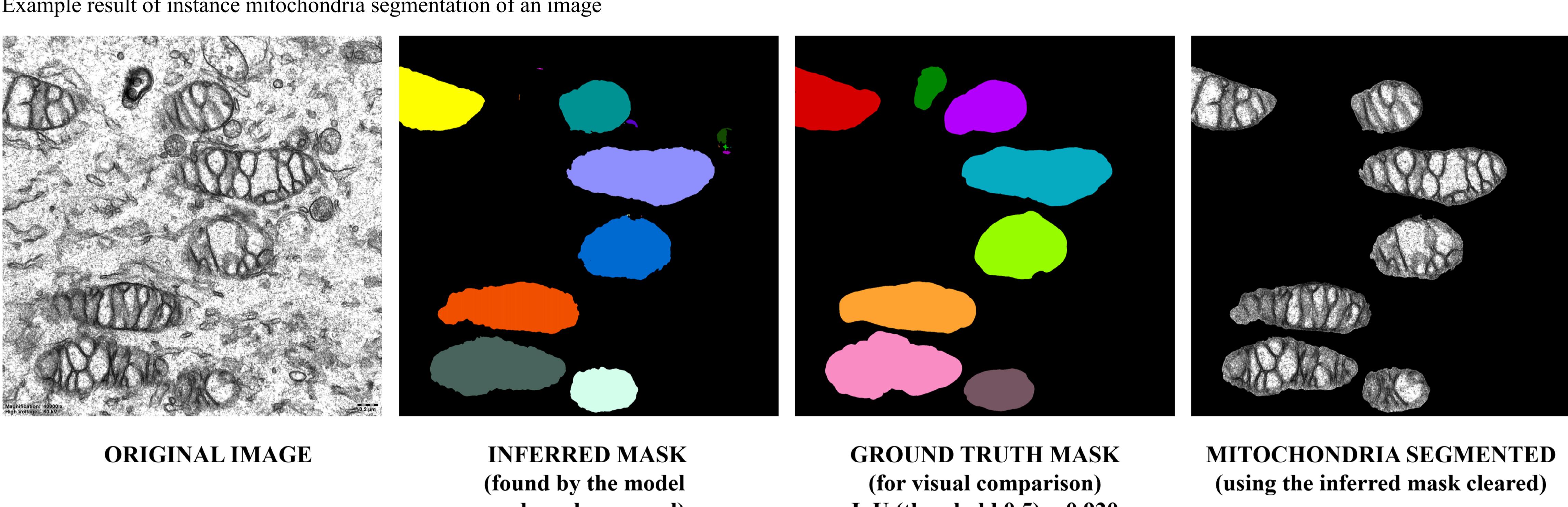
IoU = 0.518

IoU (0.5) = 0.672

Conclusion: We have developed a two-step deep learning process for classifying mitochondrial morphology and cristae from high-resolution EM images. This approach allows for scalable and reproducible mapping of cristae morphology, detecting repeating structural abnormalities such as cristae loss and onion ring formation, which are associated with specific mitochondrial dysfunctions.

By linking deep learning-based image analysis with mitochondrial pathology, our framework provides a powerful tool for advanced diagnostic interpretation and mechanistic understanding of mitochondrial diseases.

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STEP 2: Segmented mitochondria → Segmented and classified cristae

Example result of panoptic cristae segmentation and classification of an image

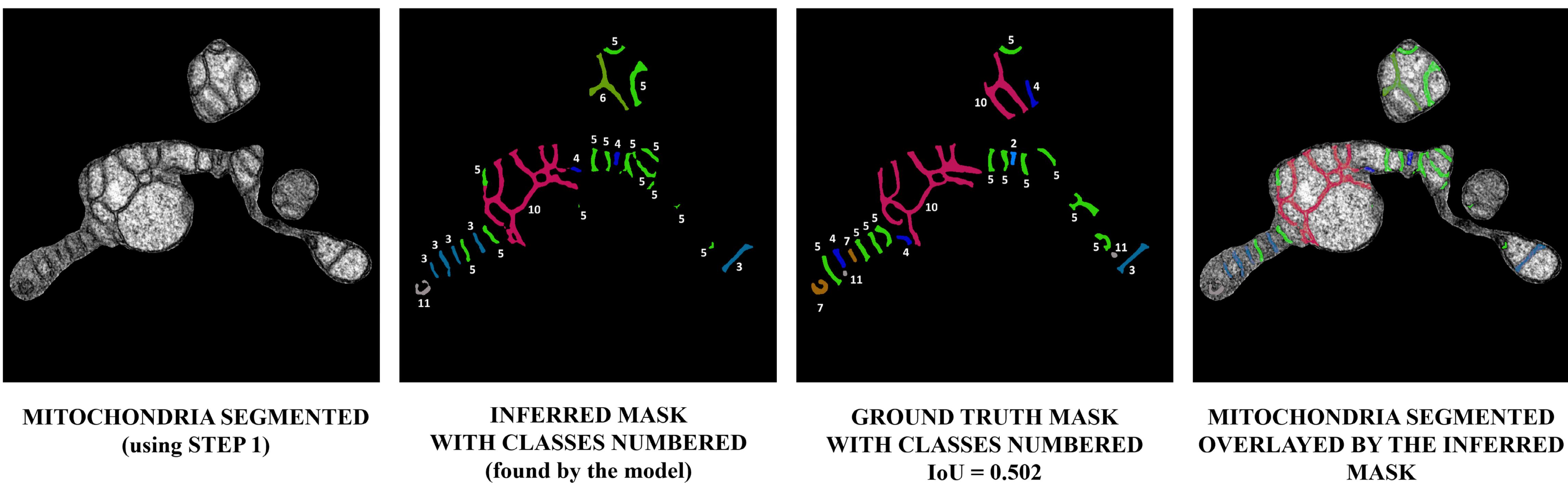


Table with cristae morphological classes and their corresponding numbers

Outer membrane	Lamellar						Globular						Onion shape	Junction
	Straight	Tilted	Disconnected	Connected	Disconnected									
One junction	Double junction	One junction	Double junction	Triple junction	No junction	One junction	Double junction	Multi junction	No junction	Label11	Label12	Label13		
Label1	Label2	Label3	Label4	Label5	Label6	Label7	Label8	Label9	Label10	Label11	Label12	Label13		

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