

Enhanced Large-Area TEM Analysis on Mitochondrial Alteration in Brain Neuronal Cell of Parkinson's Disease Model Mouse Using Montage Function Installed in JEM 1400-Plus System

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Despite the important role of transmission electron microscope (TEM) in providing tremendous ultrastructural information of cells, organelles, and molecules [1], one of the disadvantages of TEM is the difficulty associated with observing large-area with high resolution at low-magnification [2 and 3]. Although several analytical techniques and equipments including ultramicrotomy-combined serial block face scanning EM have been developed recently to overcome such problem, those are mostly concentrated in SEM applications [1, 2, 4, 5, and 6]. In this research, to acquire large-area TEM images with high-resolution, we used the montage function in TEM Center, a software suite that controls JEM 1400-Plus TEM as described in Figure 1. Then, we compared the resolution of low mag. reference image (4,000X) acquired using normal imaging mode with that of 3X3 montage image (12,000X). The spatial resolution of montage image was improved without any image distortion during montage process (Figure 2). Next, using the montage function, we focused on the morphological alterations of the mitochondria in substantia nigra of hLRRK2 transgenic (G2019S) Parkinson's disease model mice. Normal mitochondria in cell body and axon of wild-type mice mostly contained well-conserved ultrastructure. They maintained dense matrix, compacted with thin and uniform cristae, and surrounded by clear inner and outer membrane. However, majority of mitochondria in cell body and axon of the substantia nigra of hLRRK2 transgenic mice were swollen, different from the structure found in wild-type mice. Also, the mitochondria have deteriorated structure, with the cristae severely disrupted through the entire mitochondrial lumen, and the inner and outer mitochondrial membranes were partially disappeared. This result clearly shows the complex structural alteration of mitochondria in brain neuronal cells of Parkinson's disease model mice, and demonstrates the contribution of montage-based large-area TEM image analysis system for the acquisition of high throughput statistical information for an accurate interpretation of various morpho-functional alterations of cellular organelles [7].

References:

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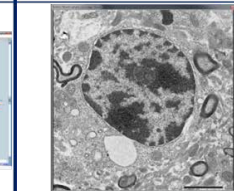

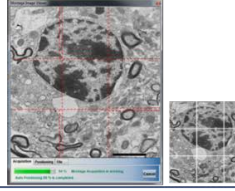
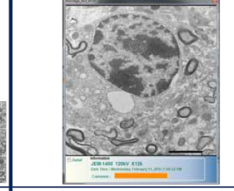
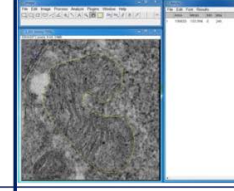
1. Sample preparation	2. JEM 1400-Plus (JEOL)	3. TEM Center (JEOL)	4. Selecting montage area
 <ul style="list-style-type: none"> • Ultramicrotomy • Collection of sections on grids • Double staining with U and Pb 	 <ul style="list-style-type: none"> • Specimen loading into TEM • HT voltage: 120 kV • Beam alignment and focusing 	 <ul style="list-style-type: none"> • Starting the TEM Center system for JEM 1400-Plus operation • Control the parameters for montage function 	 <ul style="list-style-type: none"> • Selecting region of interesting area for TEM imaging at low mag. (e.g., 4,000 X) • Acquire the reference image
5. Calibration step	6. Reconstruction step	7. Montage completion	8. Image analysis
 <ul style="list-style-type: none"> • Calibration of exact spot of image can guide the X, Y axis during montage process by auto or manual calibration mode 	 <ul style="list-style-type: none"> • Acquire and reconstruct the pieces of montage (3X3 in this figure) image with low dose at higher mag. (e.g., 12,000 X) 	 <ul style="list-style-type: none"> • Acquire montage image 	 <ul style="list-style-type: none"> • Analysis of acquired image using Image J or other analytical software

Figure 1. Outline of the experimental procedure for the enhanced large-area EM image acquisition using montage function in TEM Center of JEM 1400-Plus TEM. Simplified flowchart explaining the main experimental steps and conditions for the montage process is described.

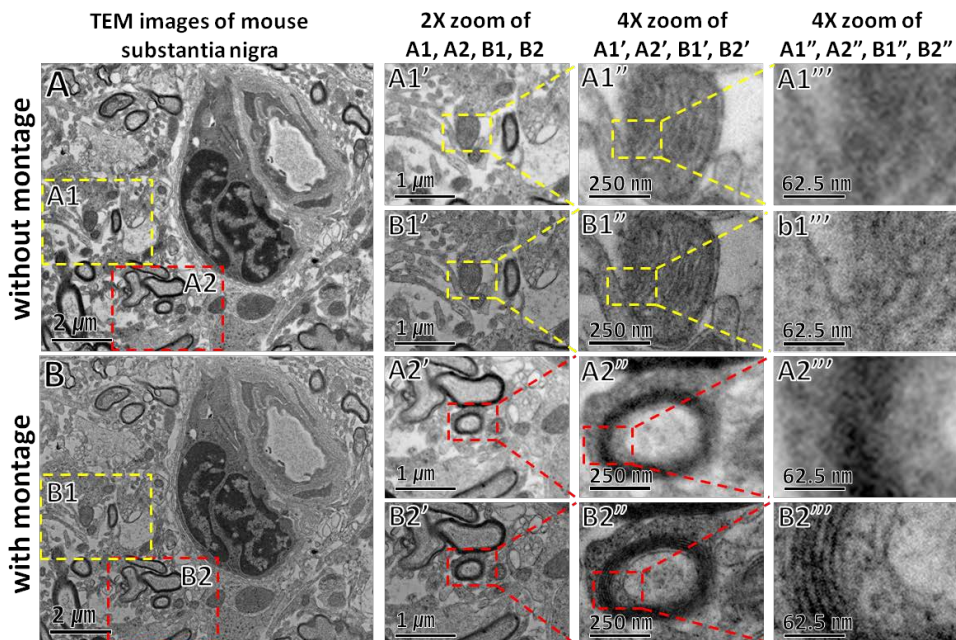


Figure 2. Improvement of the spatial resolution after montage process. The images of mouse substantia nigra without (A) or with (B) montage from same large-area are shown, respectively. **A1'-B2'**: 2X zoomed image of A1-B2. **A1''-B2''**: 4X zoomed image of A1'-B2'. **A1'''-B2'''**: 4X zoomed image of A1''-B2''.