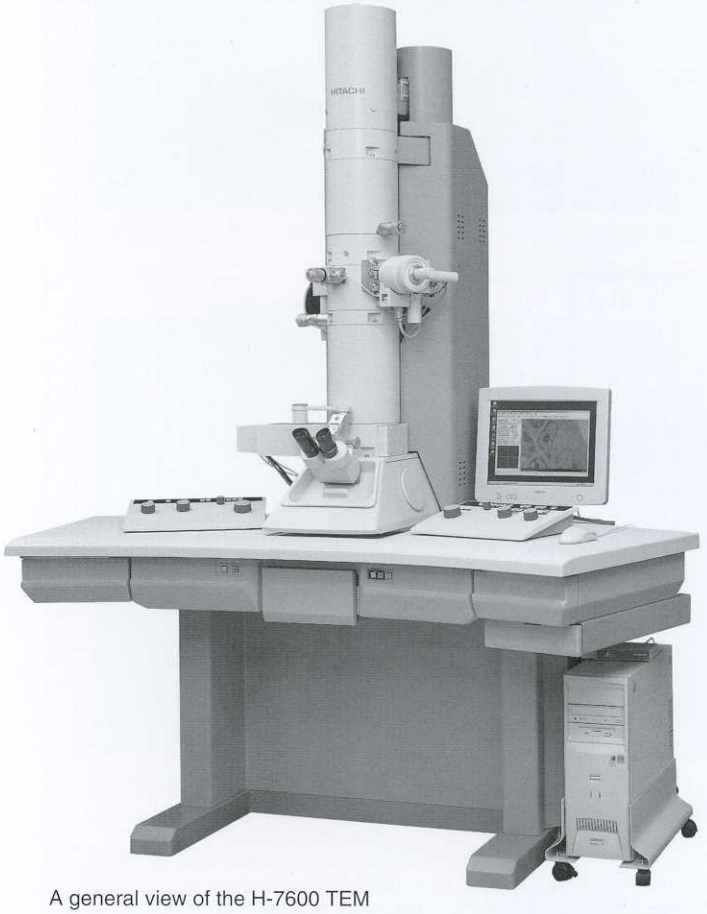
HITACHI SCIENTIFIC INSTRUMENT
TECHNICAL DATA
TEM

SHEET NO. 119

SUBJECT: THREE DIMENSIONAL OBSERVATION OF FUNCTIONAL PROTEIN USING AN AUTOMATED SPECIMEN TILTING AND RECORDING FUNCTION

INSTRUMENT: THE H-7600 TRANSMISSION ELECTRON MICROSCOPE

CONTENTS



A general view of the H-7600 TEM

1. INTRODUCTION

Thanks to advancements in genome analysis, genetic information of various proteins is being resolved these days. For better understanding of the functions of proteins, 3 dimensional structure analysis has become an important research objective. High resolution electron microscopy which allows protein observation in molecular levels has, therefore, been considered an important research technique.

Sequential sectioning and stereoscopic techniques have been employed for 3 dimensional observations using electron microscopes. Recently, computer tomography (CT) techniques, which allow image processing after observation of sequential specimen tilt images and 3 dimensional image reconstruction, have been utilized.

For reconstruction of quality images using the electron beam CT, acquisition of a few ten to a few hundred images using a sequentially tilted specimen at large angles is required. In addition, shift of images due to specimen tilting operation needs to be corrected accurately.

We have developed a new function for the H-7600. It allows sequential specimen tilting and corresponding image recording automatically. It also allows 3-dimensional observation. We have tested this sequential image acquisition using a functional protein for 3-dimensional image reconstruction and observation. We report here on the principle and some initial results.

2. AUTOMATED SEQUENTIAL SPECIMEN TILTING AND IMAGE RECORDING

Fig. 1 shows a system construction of the H-7600 with an automated specimen tilting function. The H-7600 has Windows® PC, CCD-camera for high speed image processing, and an FPD-monitor in its standard configuration. The new automated specimen tilting and image recording function uses an additional slow scan CCD-camera for image recording at much higher pixel density.

Table 1 shows specifications of the new automated specimen tilting and recording function. The eucentric specimen goniometer stage allows specimen tilting from +60° to –60° at 0.5°/step continuously and corresponding image recording.

Fig. 2 shows a basic operating monitor display for high angle specimen tilting with the H-7600. The left-hand circle shows the stage traverse area and + mark the current stage position. On the right, X–Y coordinates of the current stage position and the specimen tilting angle are shown. Operators can select various specimen holders including the ±60° tilt holder simply by clicking "Holder" in the menu. Fig. 3 shows a monitor display for setting the specimen tilt conditions when the ±60° tilt holder is selected.

Fig. 4 shows an operating flow for the automated specimen tilt and image recording function. Using Fig. 3, conditions for the sequential specimen tilt such as angles/step, range of angles, etc. are set as explained in the above. After setting these conditions, start the system.

The specimen stage is automatically tilted and the tilted image is recorded by the CCD-camera for high speed image processing. Based on this image, focus conditions and specimen positions are corrected. The automated sequential specimen tilting and image recording function of the H-7600 uses a unique phase-only-correction for these corrections. It performs accurate corrections without problems for any change of image brightness. After these corrections, quality images are automatically acquired by the slow scan CCD-camera at higher pixel density. A series of operations from stage tilting and various corrections to image acquisitions is performed automatically and sequentially for a preset range of specimen tilt angles.

Table 1 Specifications of the automatic specimen tilting function

Specimen stage	Eucentric specimen goniometer stage
Tilting range	−60° ~ +60°
Minimum tilt angle for setting	±0.5° (±0.1°)

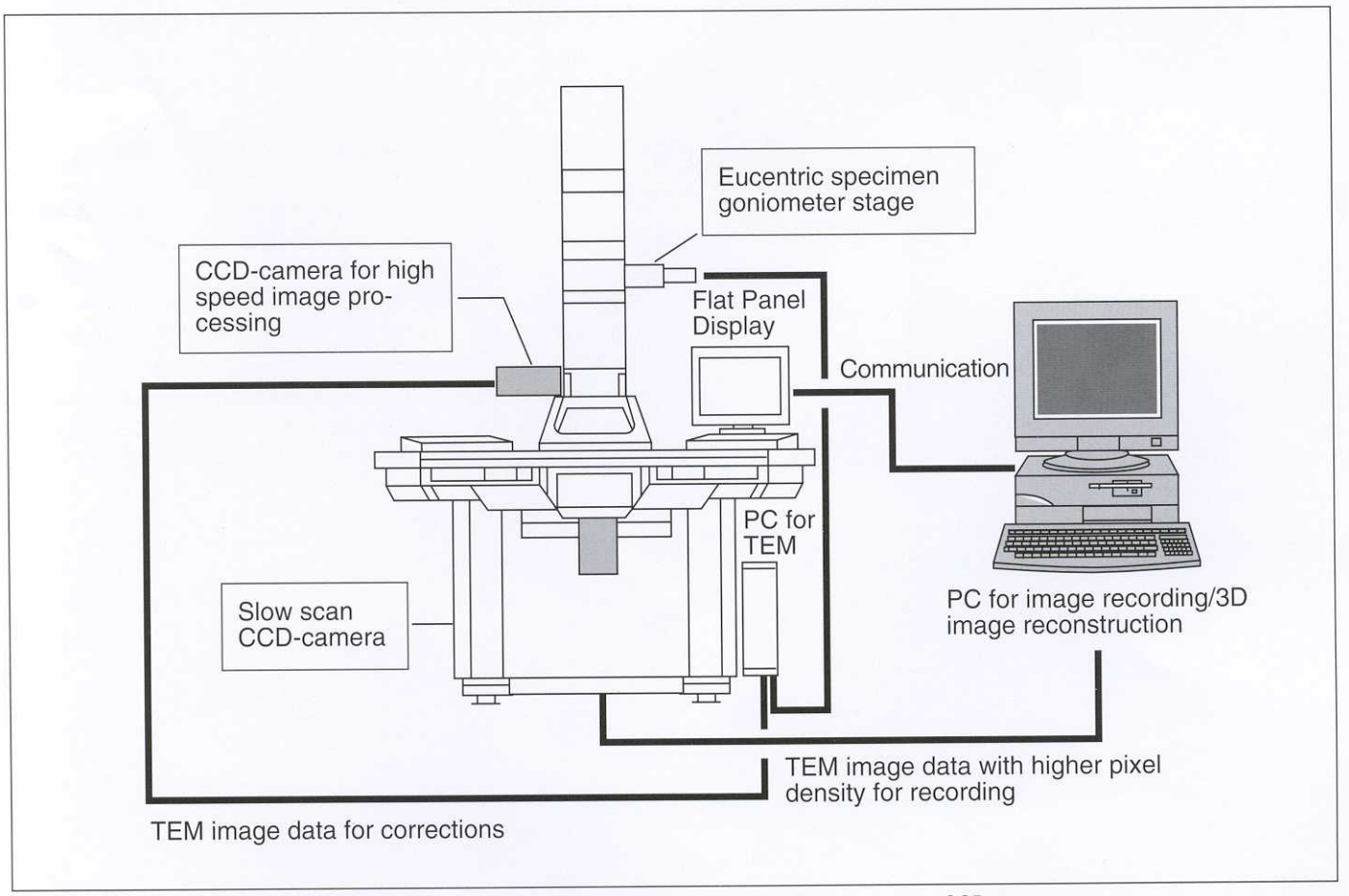


Fig. 1 System configuration of the H-7600 with the automatic specimen tilting function and a slow-scan CCD-camera

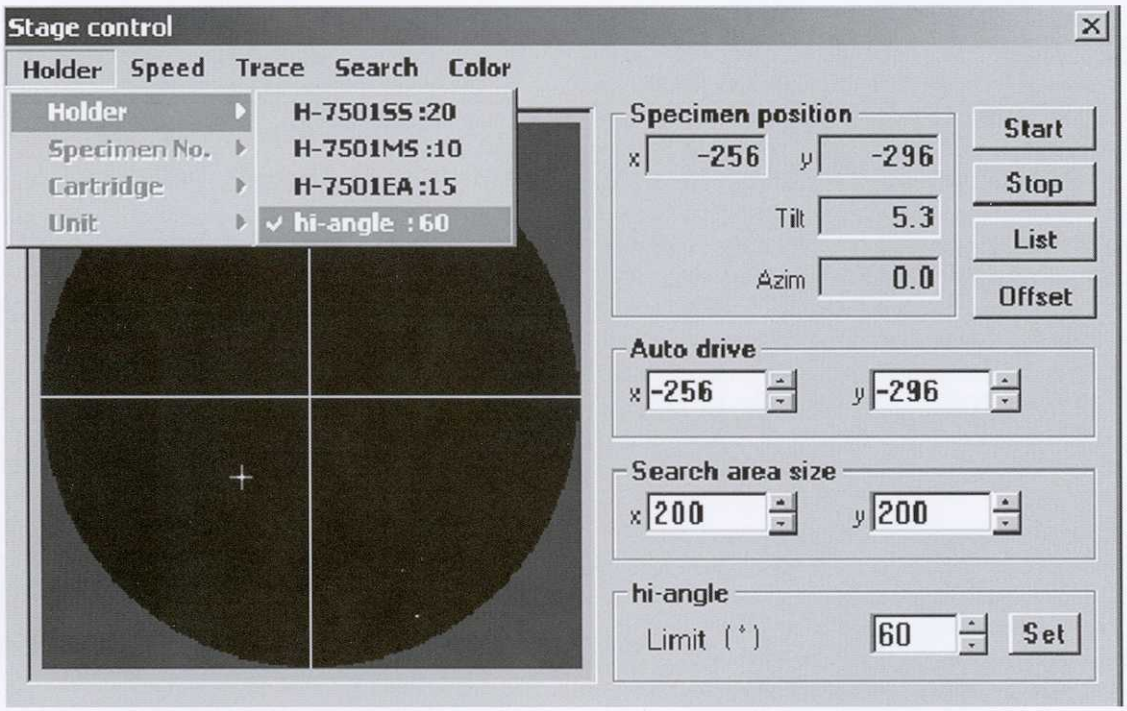


Fig. 2 A graphical user interface (GUI) display for selection of specimen holders including a high-angle tilting specimen holder

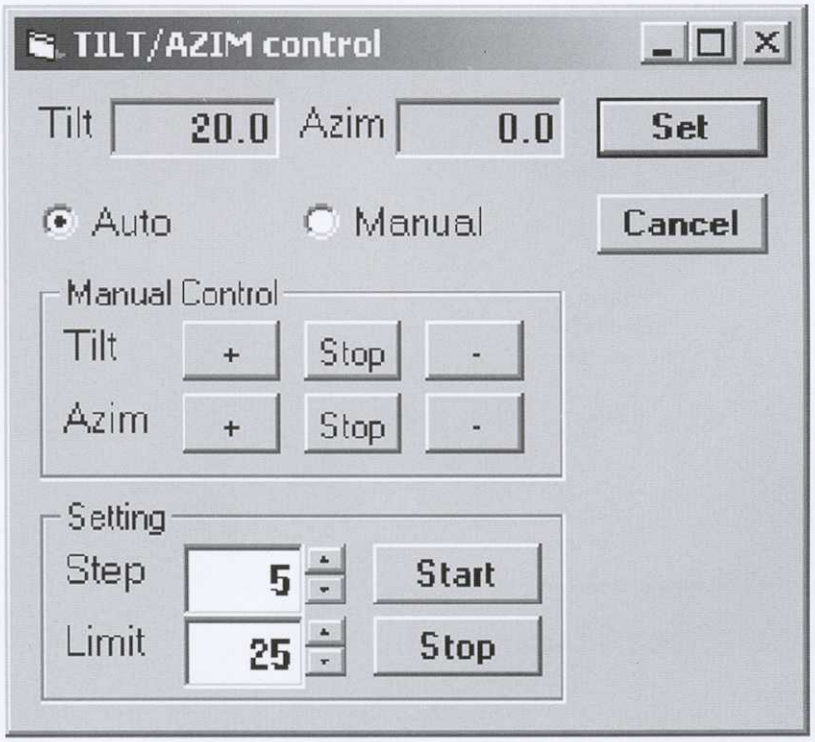


Fig. 3 GUI display for setting conditions of the automatic specimen tilting function

3. SOME INITIAL OBSERVATIONS OF BIOLOGICAL SPECIMENS USING THE AUTOMATED SEQUENTIAL SPECIMEN TILTING AND IMAGE RECORDING

Fig. 5 shows a transmission image of bovine cerebellum, prepared by a freeze-fracture deep-etch and replication. The cerebellum is an element of the central nerve for spine animals. It is an important organ for keeping a good balance of the body or correct conditions of muscle tissues.

It contains various receptors that are needed for signal transmission from nerves. This is a micrograph showing one of such receptors called inositol-1,4,5-triphosphate receptor molecules[1]. Fig. 6 shows a part of transmission image of vesicles recorded at 3°/step for a range of ±60°. In the vesicular structure, there are regularly arranged receptors (shown by an arrow) made clearly visible. By comparing images of -9° and +21° tilt in particular, you may note that individual receptors in the regular arrangements look clearly different.

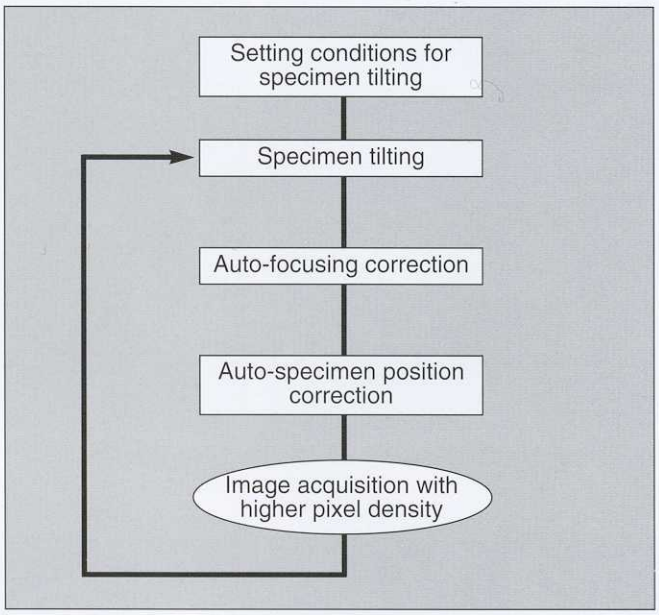


Fig. 4. Operation flow for a sequential specimen tilting with the automatic specimen tilting function

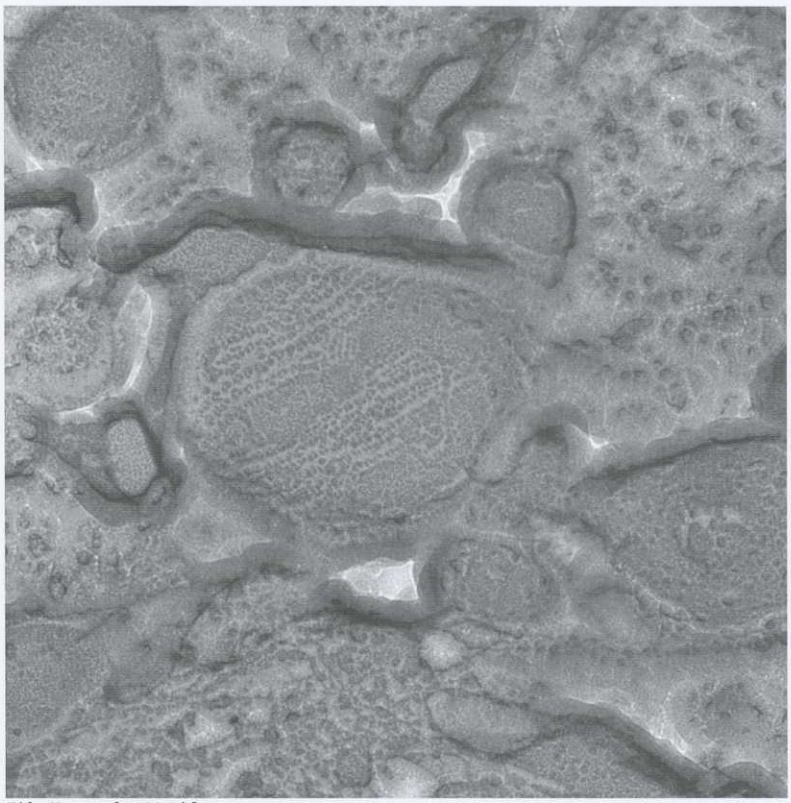


Fig. 5 A transmission electron microscope (TEM) image of a quick-freeze deep-etched replica of bovine cerebellum
Direct magnification: ×20,000 Accelerating voltage: 100 kV

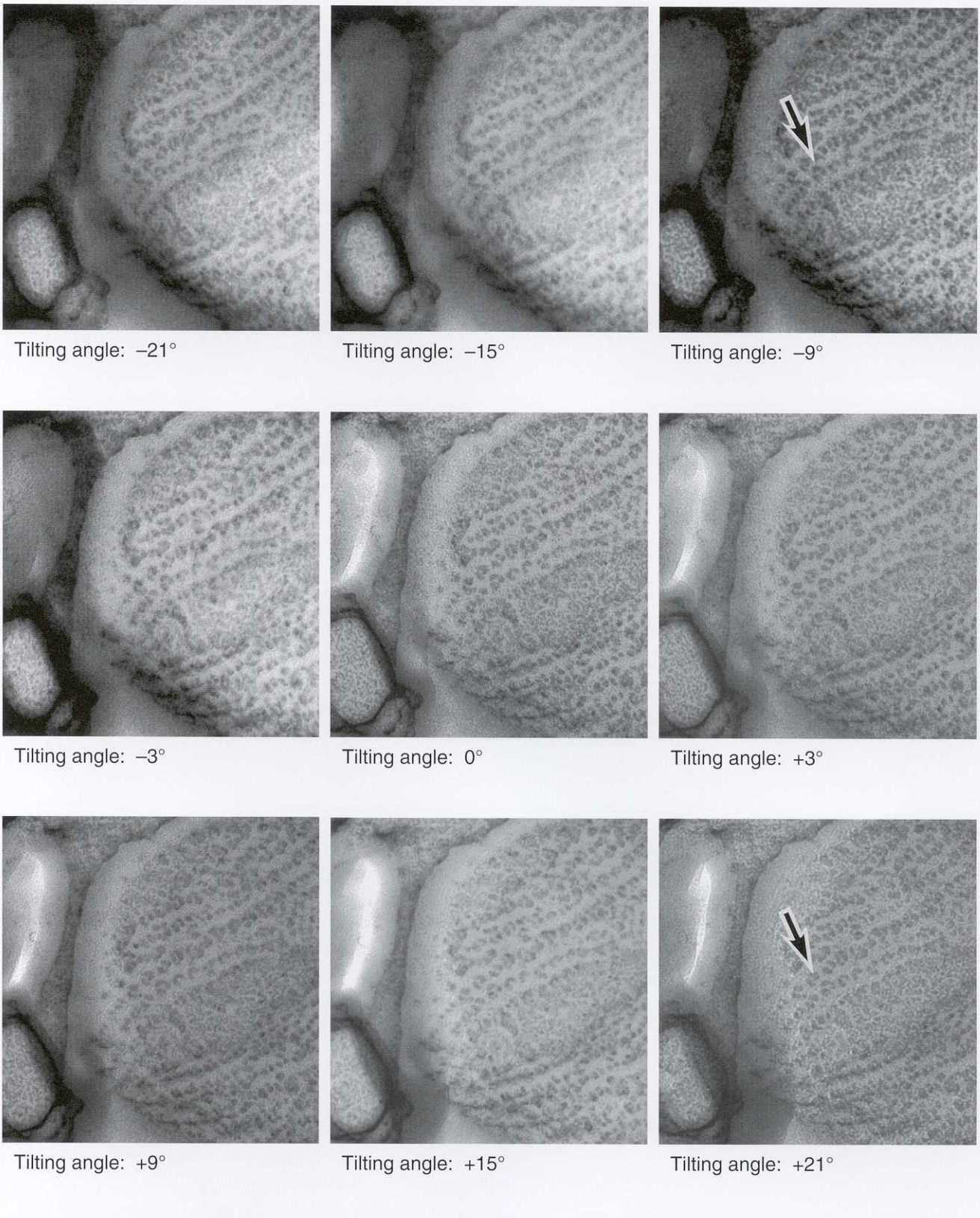


Fig. 6 Sequentially tilted TEM images of a quick-freeze deep-etched replica of bovine cerebellum
Direct magnification: ×70,000 Accelerating voltage: 100 kV Specimen tilt: –60° ~ +60° (Tilting step: 3°/image)

4. CLOSING REMARKS

We have introduced a new automated specimen tilting and image recording function and some initial applications with nerve tissues prepared by a quick-freeze-replica technique. In the research field of structural biology, electron microscopy is of high interest for its high resolving power as one of the 3 major measurement techniques which include NMR and X-ray diffraction. High resolution image observation of functional proteins using a sequential specimen tilting function is a typical application of electron microscopes. Three-dimensional observation using electron microscopes is one of the very important techniques to determine fine structures of biological materials in molecular biology and other biological fields. We wish to thank Prof. Eisaku Katayama, Division of Biomolecular Imaging, Institute of Medical Science, The University of Tokyo for providing precious specimens.

References

1) E. Katayama et al., Native structure and arrangement of inositol-1,4,5-triphosphate receptor molecules in bovine cerebellar Purkinje cells as studied by quick-freeze deep-etch microsccopy. The EMBO Journal, 15, 4844-4851 (1996)

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