HITACHI SCIENTIFIC INSTRUMENT
TECHNICAL DATA
TEM

SHEET NO. 111

SUBJECT: DEVELOPMENT OF AN AUTOMATED PARTICLE SEARCH SYSTEM FOR THE H-7600 TEM AND SOME APPLICATIONS

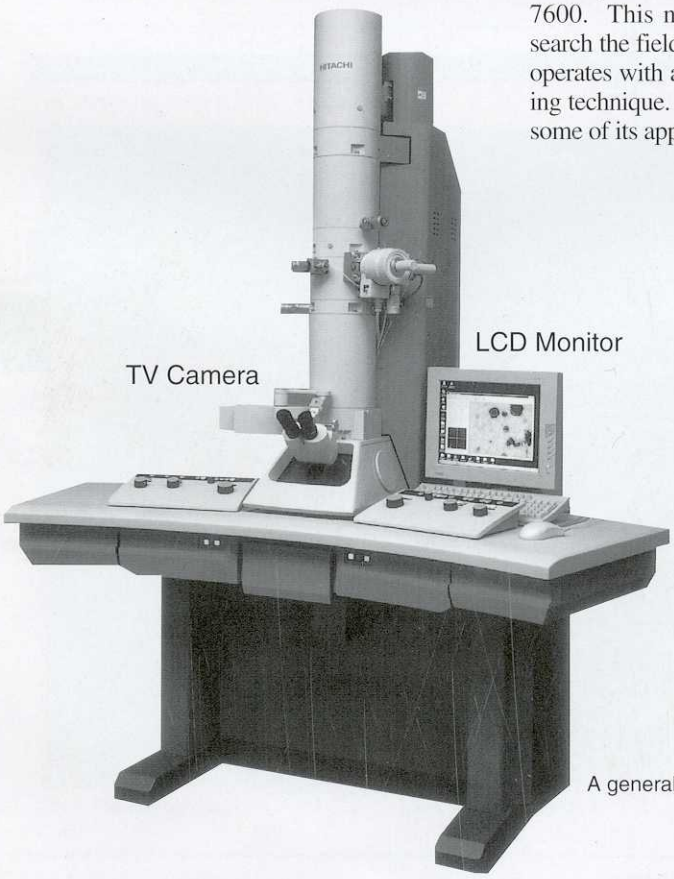
INSTRUMENT: H-7600 TRANSMISSION ELECTRON MICROSCOPE

CONTENTS

1. INTRODUCTION

We have completed development of an automated focusing system for the H-7600 TEM by incorporating a TV-camera and an image processing system. We have developed this system further so that the system may allow a series of image recording automatically, once an area (or areas) of interest is selected by the operator.

In electron microscopy the operator usually spends most of his (her) time searching an area (or areas) of interest on the grid specimen. This is not only time consuming but also requires a lot of patience on the part of operators. Recently we have completed development of an automated particle search system for the H-7600. This new system has been designed to help operators search the field of interest efficiently and effortlessly. The system operates with an image recognition system using a pattern matching technique. We are pleased to introduce you to the system and some of its applications.



A general view of the H-7600 TEM

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2. AUTOMATED PARTICLE SEARCH SYSTEM

Fig. 1 shows a general view of the H-7600 with the automated particle search system. The system is composed of the H-7600 TEM, a TV-camera, a PC, an image processing software, and an LCD monitor. The monitor displays basic operation images of the TEM and playback (TEM) images of the TV-camera simultaneously.

TEM images of the TV-camera are sent to the PC in a form of NTSC signal and are displayed on the monitor. The same signals are sent to the image processing system from the PC together with the operating magnification, accelerating voltage and other parameters of the TEM using RS-232C. The H-7600 is network compatible so that particle images and other related information can be transferred to any other systems in the network. Table 1 shows specifications of the automated particle search system.

Fig. 2 shows a GUI (Graphic User Interface) of the operating automated particle search system. The search positions are specified simply by selecting the specimen grid hole, regardless of orientations or types of grids. Fig. 2 shows an example of search of a set of 9 positions (or 3 horizontal and 3 vertical positions). The system allows a preset for 10 sets of search positions or a total of 90 holes for automated search.

This system has been designed for search of particles such as viruses so that it requires operators to specify a particle diameter and a roundness. Fig. 3 shows a GUI for setting image parameters and the status of search.

Specify the image parameters as shown by arrows. When the system starts searching, the searched areas are shown on the monitor. Images of the searched areas are displayed on the monitor in a form of thumbnail sketches.

These images are stored with corresponding acquisition parameters as shown in Figs. 4a and 4b.

Table 1 : Specifications of Automated Specimen Search System

Item	Specification
Magnification	× 8,000 or over
Searched particle size	10 nm or over
Digital image size	640 × 480 pixels (about 300 kB)*
Format of digital image	TIFF

* Image only, ~1MB: After image analysis

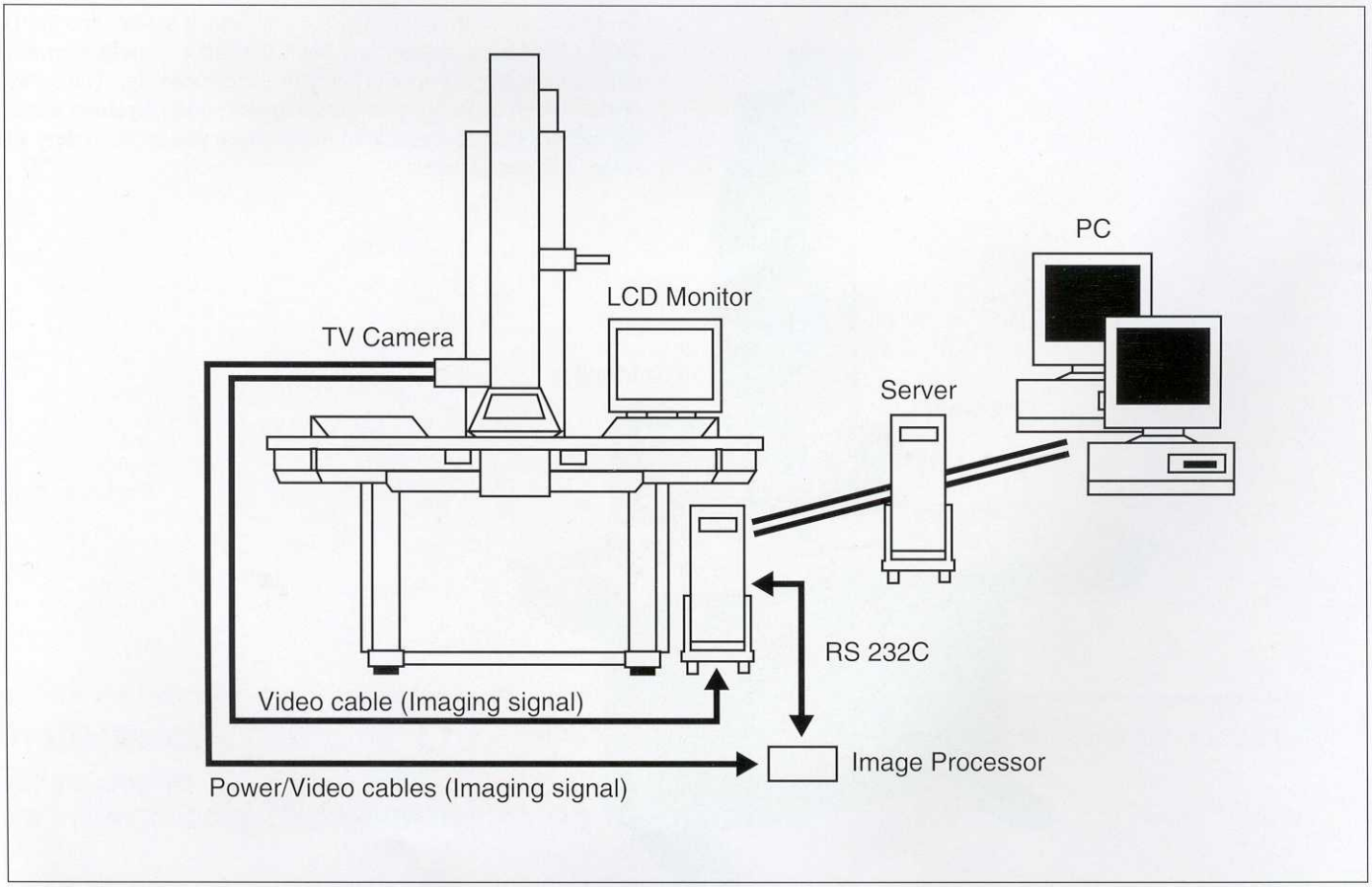


Fig. 1 The H-7600 with the automated particle search system

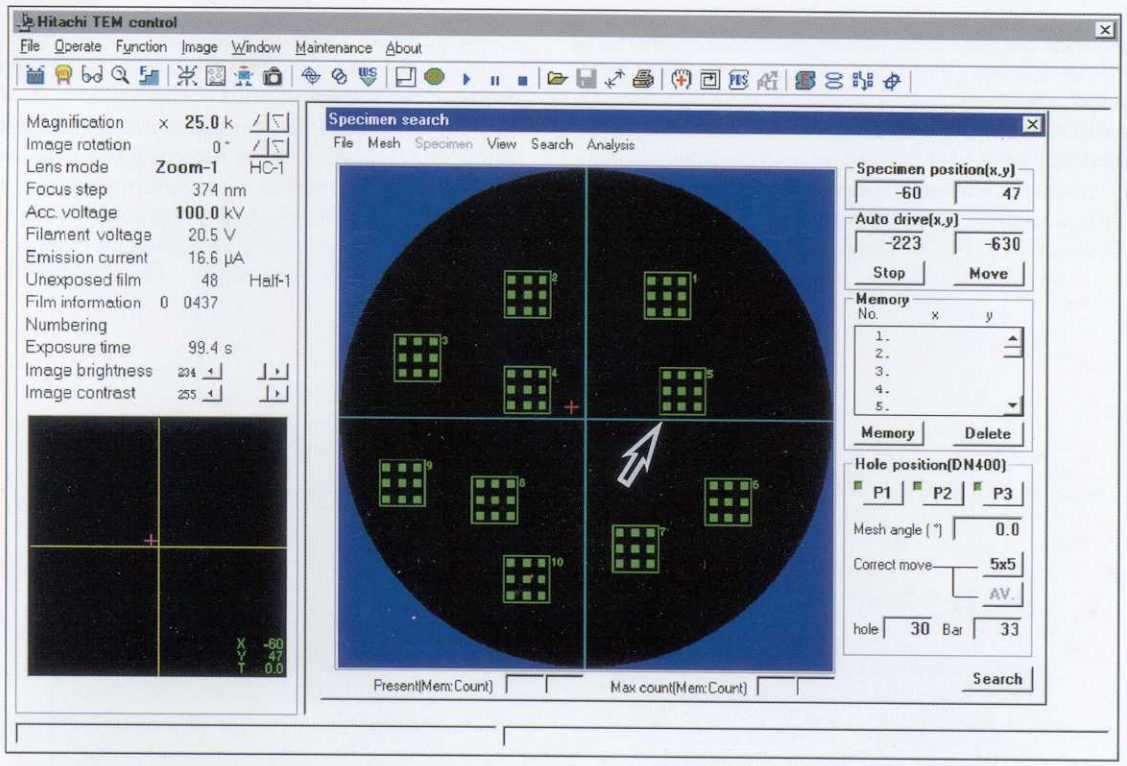


Fig. 2 GUI of the automated particle search system

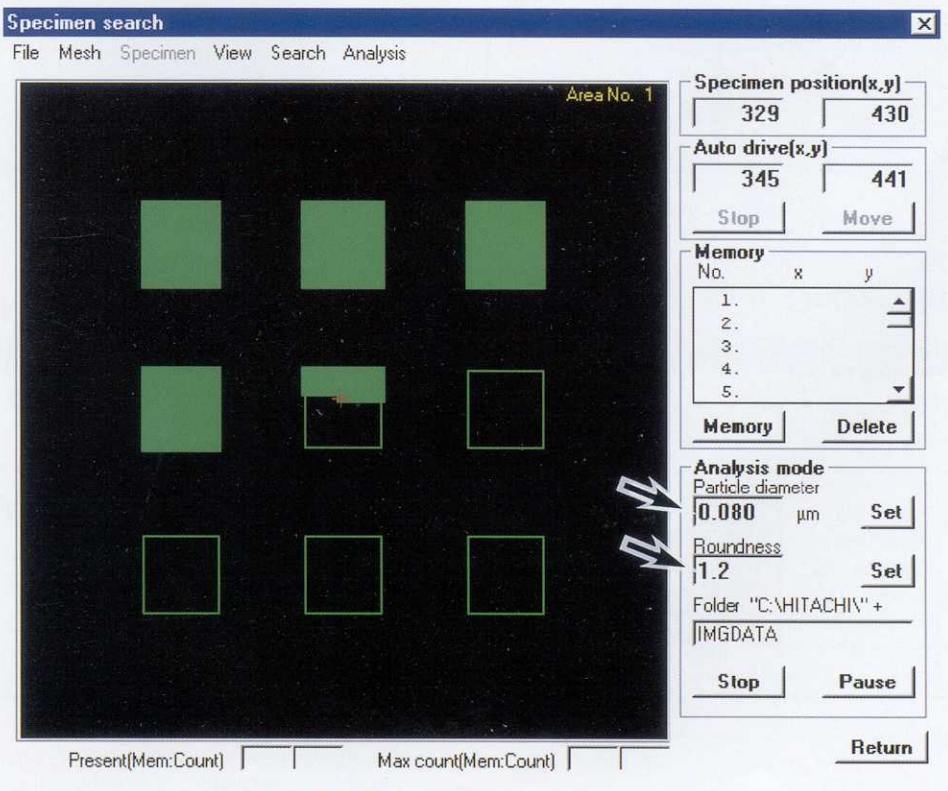


Fig. 3 Setting of parameters and search status display

3. APPLICATIONS

We have used the automated particle search system for virus particles in tissue sections. The tissue is a cultured hepatofibroblast infected with adenoviruses.

Fig. 5 is a low magnification TEM image showing a general view of the cultured cells. Adenoviruses usually exhibit icosaheral symmetry structures of about 80 nm in diameter. For searching parameters, we have used a particle diameter of 0.08 μm (80 nm) and a roundness of 1.2. Fig. 6 shows a result of the automated search.

Fig. 6a shows a searched TEM image. This image is composed of 480 × 640 pixels and is stored in TIFF format which can be used for a database.

Fig. 6b shows the same image with numbers for searched particles. Each particle has been assigned a serial number and is displayed on the monitor. We have counted 35 particles in this field of view. The H-7600 automated particle search system allows operators to specify the minimum number of particles per field depending on their needs. The searched fields are stored

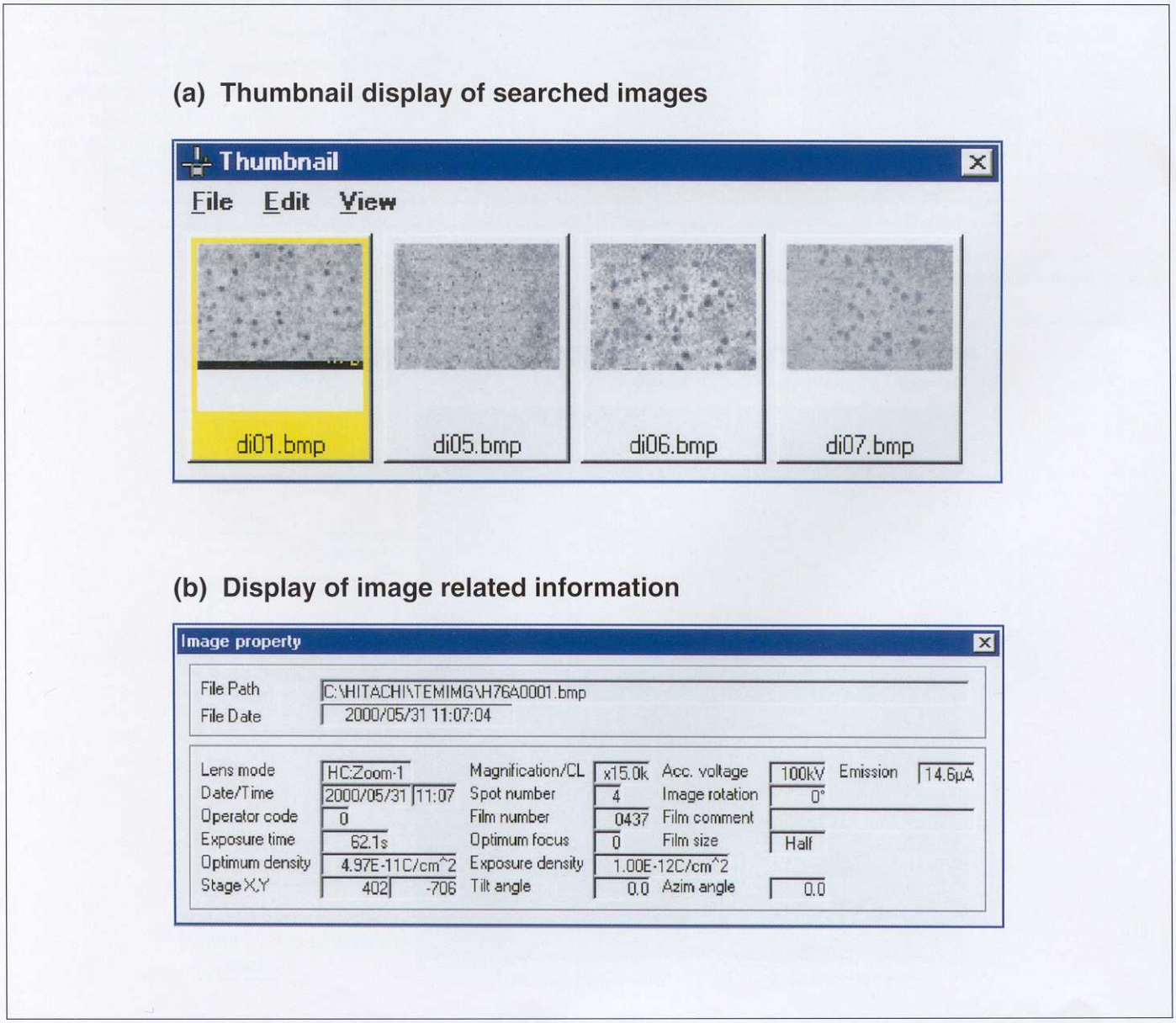


Fig. 4 Thumbnail display of searched images and image related information

with their positions so that they can be called when required at some later time, date, etc. A simple click of an image of interest on the thumbnails allows display of the corresponding image on the TEM fluorescent screen.

Fig. 7 shows a TEM image recorded using AutoPhoto Function after displaying a TEM image corresponding to a specimen position on Fig. 6.

Recording areas of the film camera are greater than those with a TV-camera (depending on the type of camera and its working positions). Reproducibility of the field is quite good as shown

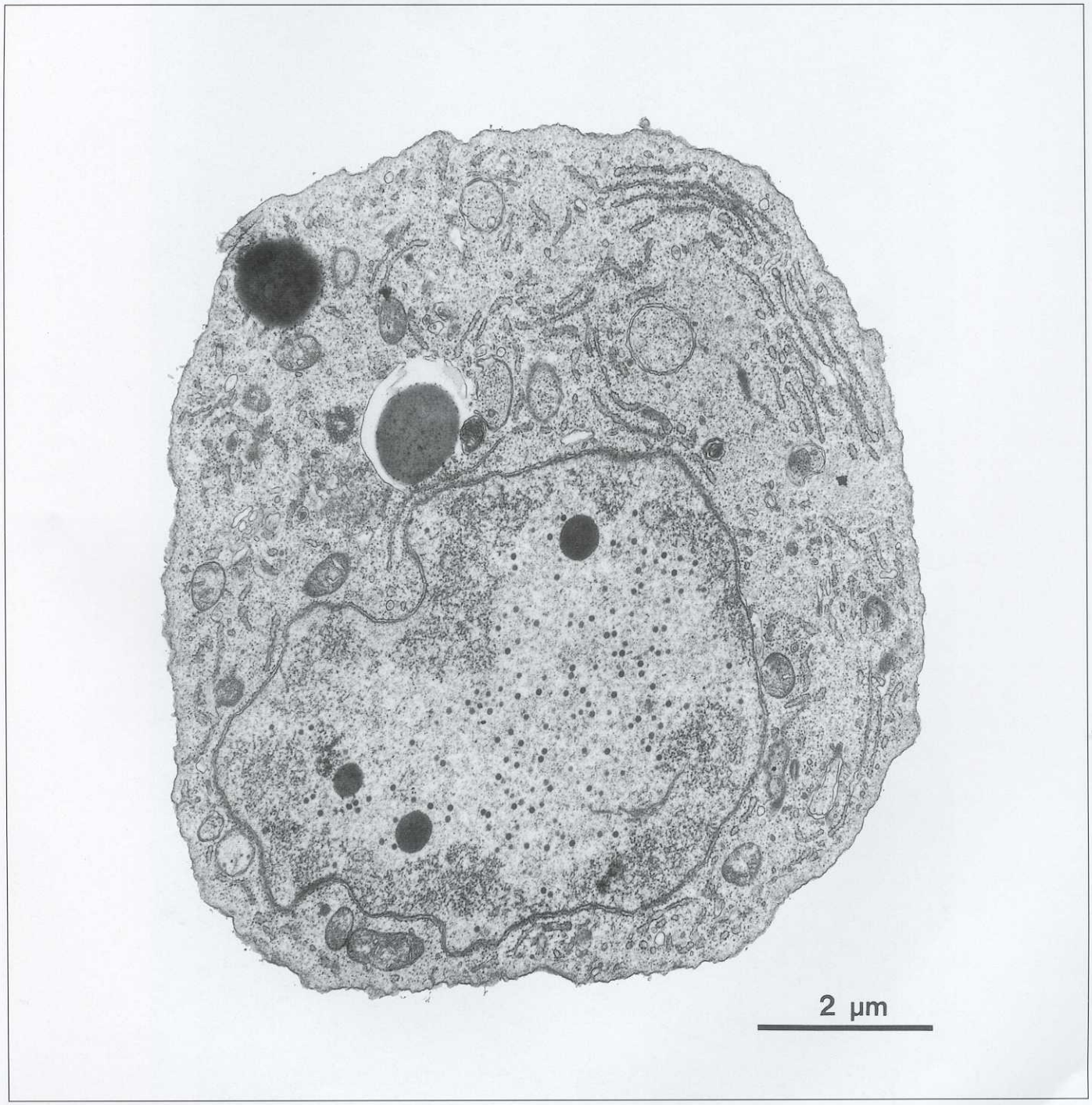
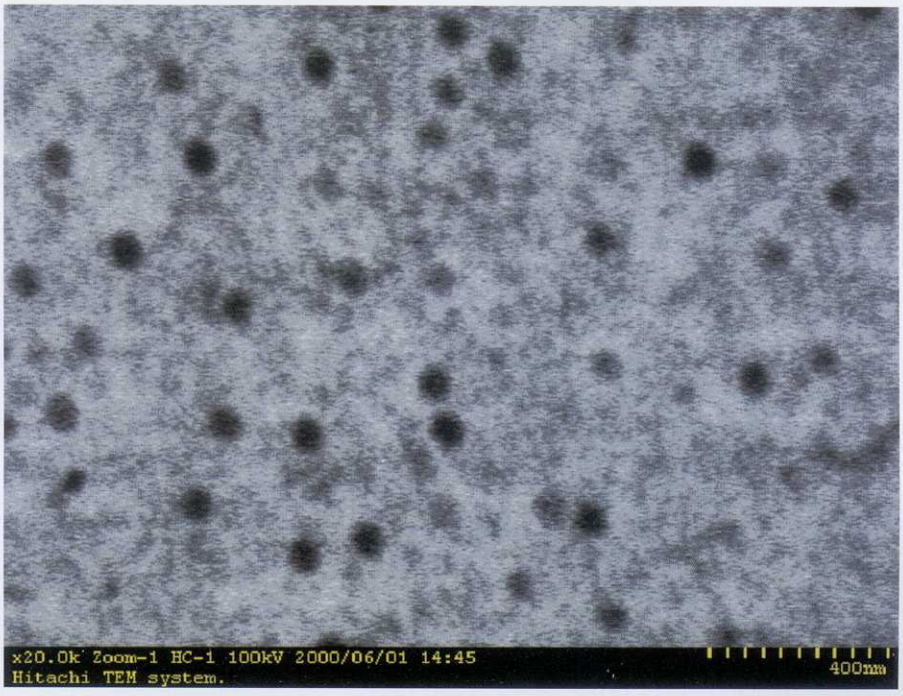


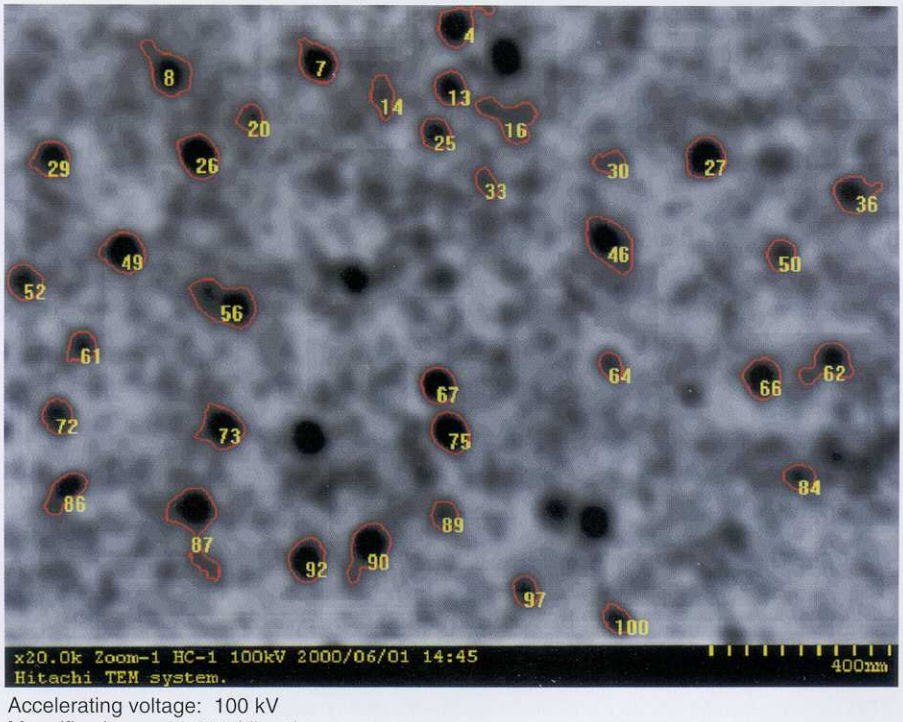
Fig. 5 A low magnification TEM image of human hepatofibroblasts infected with adenoviruses
Specimen: Resin-embedded section of human fibroblasts
Accelerating voltage: 100 kV
Magnification: ×5,000 (direct)

by Fig. 7. The AutoPhoto Function which is linked with the automated focusing allows quality image recording of individual virus particles as shown by arrows.

(a) A searched image (640 x 480 pixels)



(b) Searched particles with serial numbers (red circles) assigned for each particle



Accelerating voltage: 100 kV
Magnification: ×20,000 (direct)

Fig. 6 A typical result of the automated particle search system using sections of resin-embedded human fibroblasts infected with adenovirus

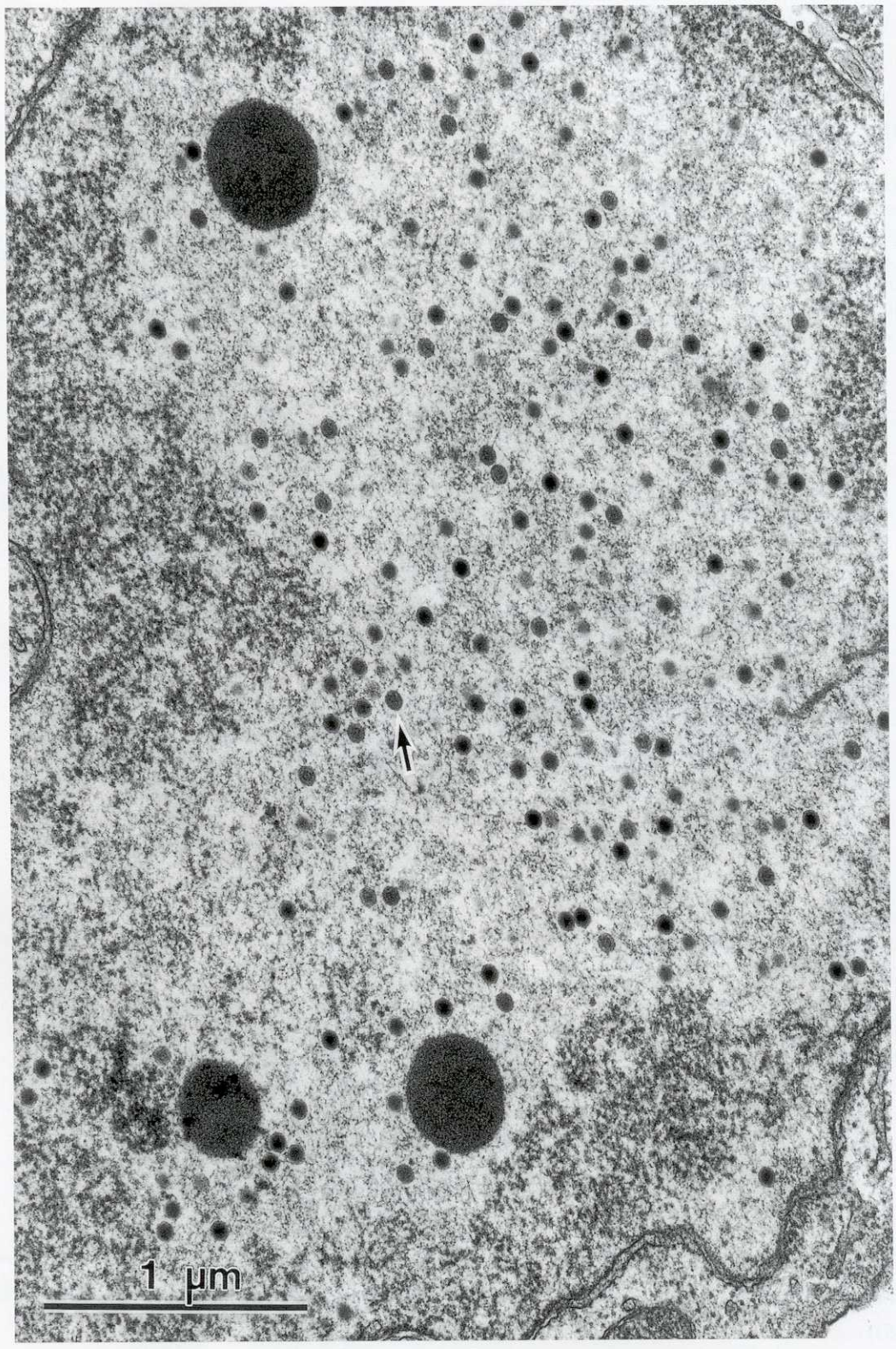


Fig. 7 A TEM image of searched particles, automatically recorded on a film using the Auto-Focus and Auto-Photo Functions (An arrow shows adenovirus)

4. CLOSING REMARKS

We have introduced the automated particle search system and some applications. The system is for particle specimens. It operates with a simple input of particle diameters and roundness. It stores searched images with corresponding specimen positions, magnification, and other TEM parameters which can be used for a database. Using the automated focusing and automated photo functions, the system allows automated image recording of searched fields. As shown by some applications, the system has high contrast and low background imaging which allows automated search of virus particles in tissue sections. We wish to develop this system further for measurement of searched particles and identification of particles by image processing. We wish to thank Dr. Satoru Fukuda, Laboratory of Electron Microscopy, Faculty of Medicine, The University of Tokyo for providing us with precious specimens.

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