

SUBJECT: USE OF IMAGE DATABASE IN BIO-MEDICAL FIELD

— USEFUL FUNCTIONS OF THE H-7600 AND THEIR APPLICATIONS —

INSTRUMENT: H-7600 TRANSMISSION ELECTRON MICROSCOPE
AMT SLOW SCAN CCD CAMERA SYSTEM

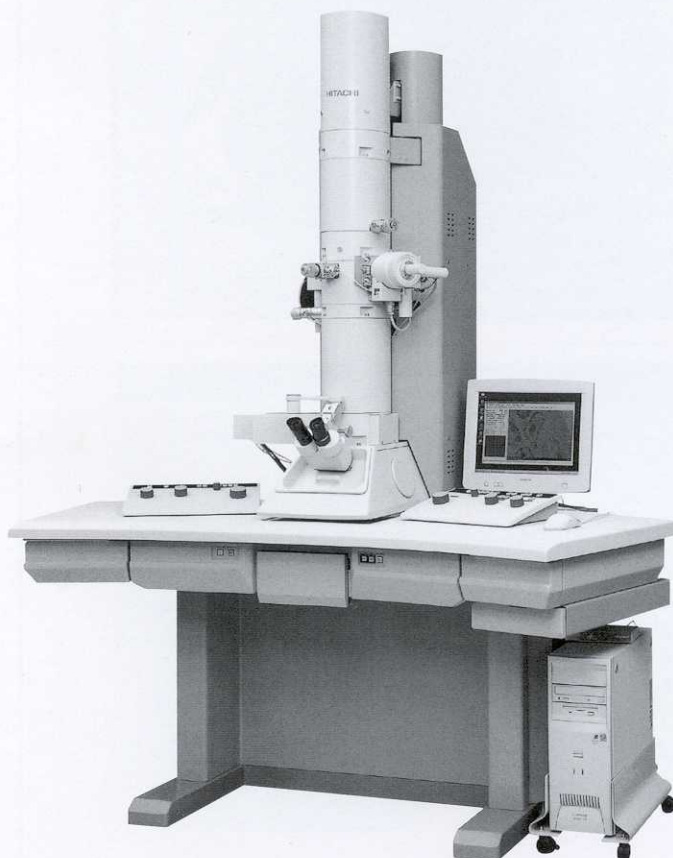
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1. INTRODUCTION

Thanks to the development of information technology (IT), it has become possible to acquire a large amount and variety of information as well as data in a speedy way. This trend has been welcomed in the field of research using transmission electron microscopes (TEMs) for the purpose of speedy use of available data. We have mounted a slow scan CCD camera on a Windows PC-TEM, the H-7600, and have made digital TEM images available on line. In addition, we have made these images available on local area network (LAN) as well as internet. In this Technical Data, we report on the use of the image database function of the H-7600 TEM and a typical application for immunological microscopy specimens with quality digital TEM images provided by them.



Configuration of Bio-TEM H-7600

2. IMAGE DATABASE OF THE H-7600

Fig. 1 shows a display of thumbnail images recorded in a digital format using the H-7600. Images are shown in Fig. 1a and their recording conditions are shown in Fig. 1b.

Fig. 2 shows a graphical user interface (GUI) of the H-7600. A TEM image recorded using AMT SSCCD camera at a video rate (512×512 pixels) is shown in GUI. This TEM image is stored in PC of the H-7600. The system allows operators to specify a directory or file and save the image quickly. The TEM

image shown here can be focused in 0.9 seconds using the auto focus function. The image can then be saved just by clicking the icon.

The thumbnail images include recorded hours and dates, magnifications, accelerating voltages and other conditions as well as files of images attached in a form of image property.

Using the image property, the H-7600 allows display of

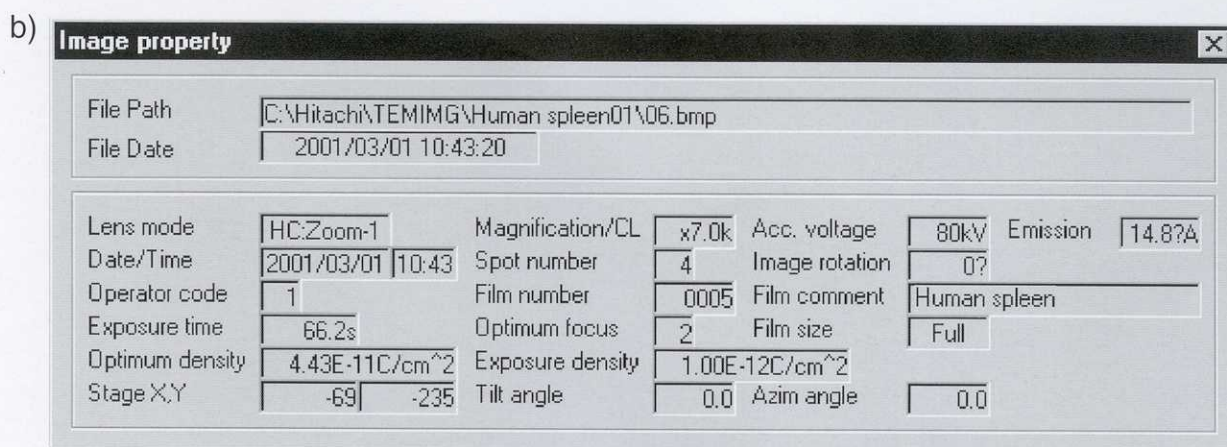
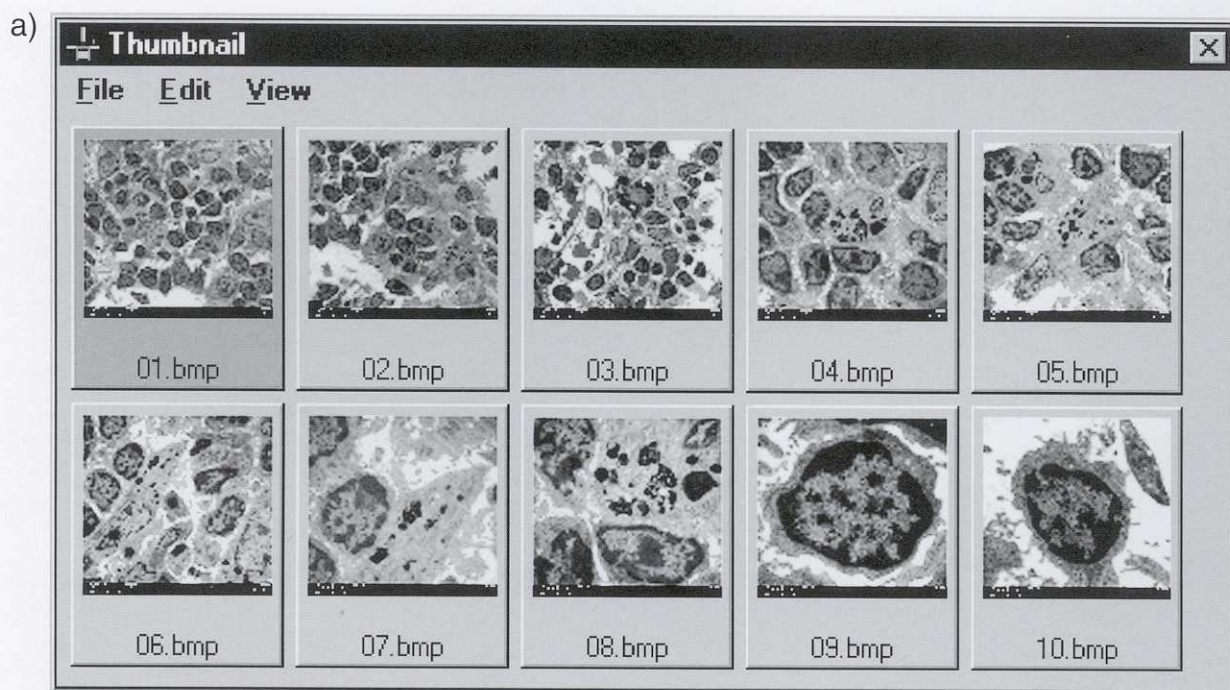


Fig. 1 Thumbnail of digital TEM images (video rate 512×512 pixel)

(a) Database of digital TEM images, (b) Image property

recording conditions of stored images. It also allows automated operation of the specimen stage back to the recorded positions of any stored images. Up to this time, it has been difficult to record the same specimen area once recorded at the same conditions. The H-7600 permits repeated specimen recording of the same field of view without problems. It allows the field once recorded in a digital format to be recorded again using conventional silver-halide films at a later time or date or the other way around.

3. NETWORK FUNCTION

Fig. 3 shows a typical example for networking of the H-7600. The H-7600 is totally network compatible including the main PC, CCD camera control, and other peripheral equipment. Through the network, digital TEM images can be stored in servers, or printed using high quality printers. These images may be accessed through terminal PCs and utilized for various purposes.

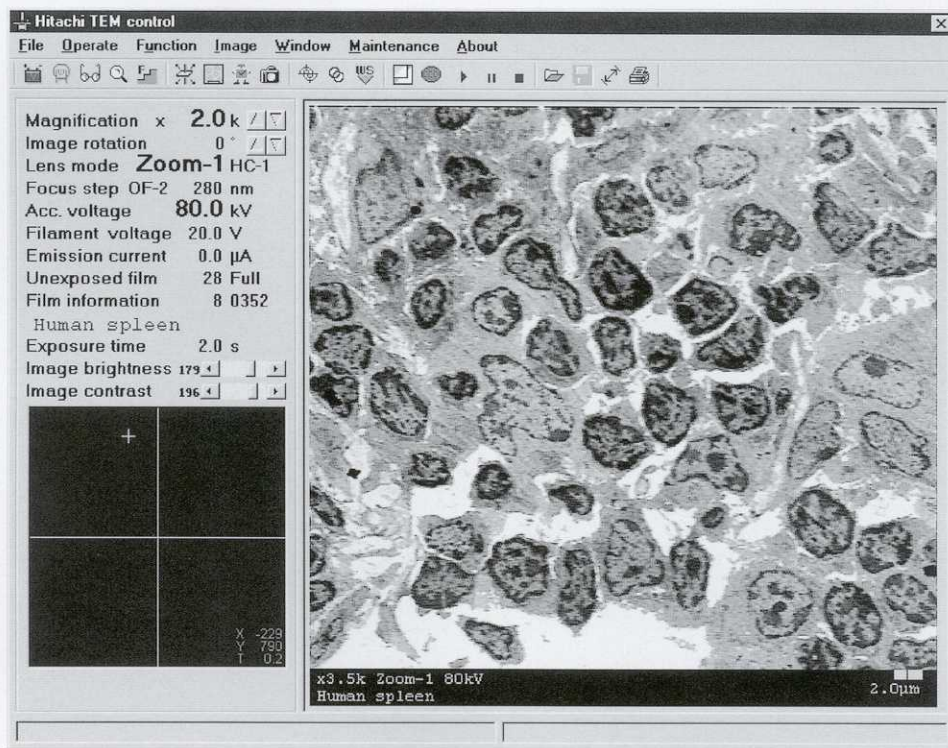


Fig. 2 Graphical user interface/GUI for the H-7600

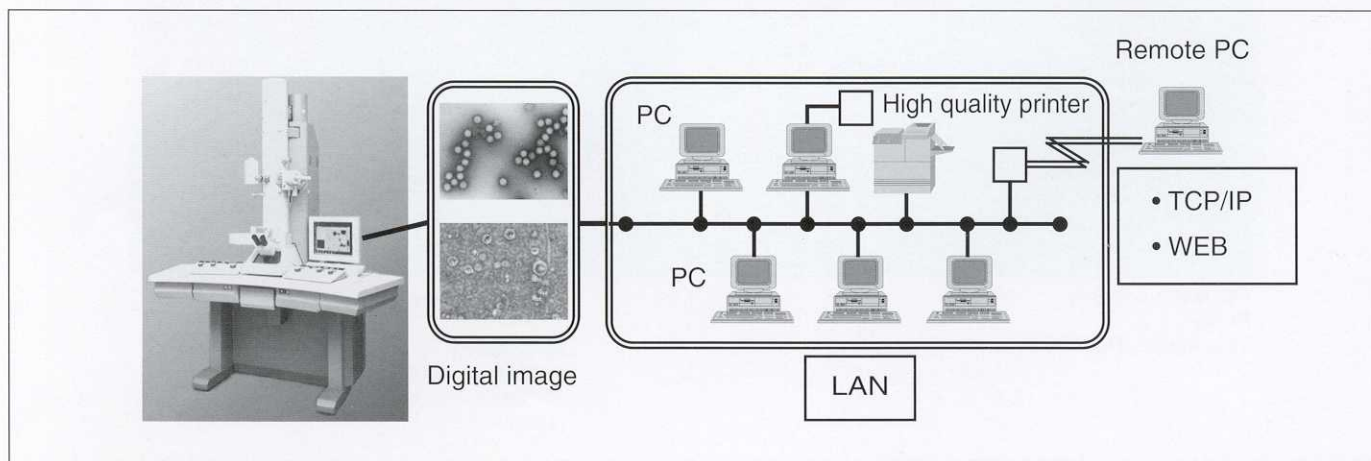


Fig. 3 Networks of H-7600 and other PC

4. APPLICATIONS

In the bio-medical research field, genome analysis has made rapid progress these days and identification and structural analysis of functional proteins are actively practiced. Immunoelectron microscopy which utilizes an antigen-antibody reaction is useful for identification, and ultrastructural distribution of proteins is an important technique. Prompt exchange of information among research scientists will be an important issue. The use of a data-

base, therefore, will become even more important in the future.

We report on a typical example of TEM image database using immunological microscopy specimens with quality digital TEM images. Small fragments of the stomachs of Wistar rats frozen at high pressure were utilized for the detection of immunoreactivity of anti-pancreatic phospholipase A2.



File Name = 01S.tif
PLA2
TEM Mode = HC-ZOOM

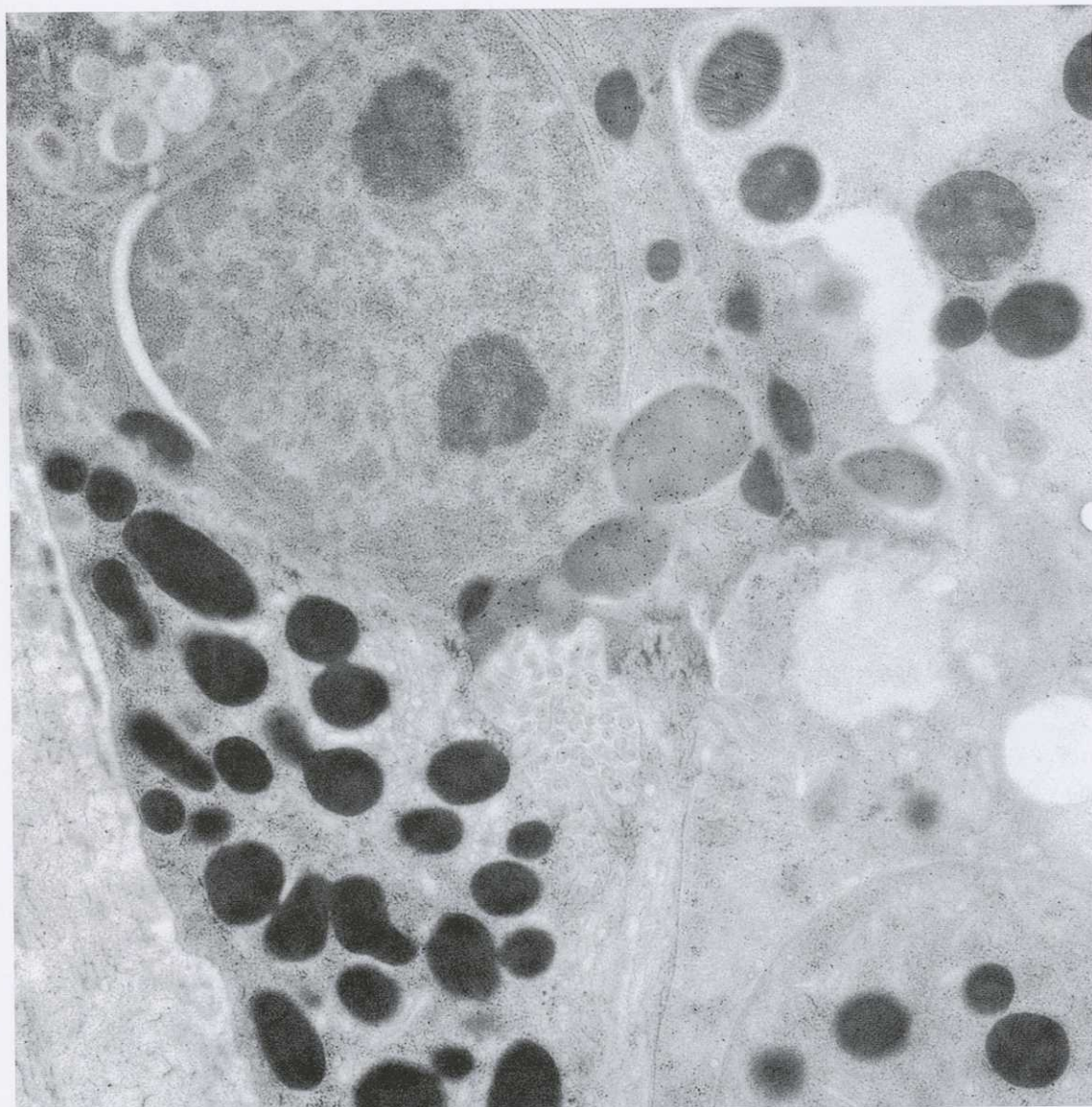
10 microns
HV = 100 kV
TEM Mag = 3500 ×
X = 290.7 Y = 304.7
H-7600

Fig. 4 Applications of digital TEM images —1—

Sample: Rat stomach, Acc. volt.: 100 kV, Direct magnification: × 3,500

Fig. 4 shows a digital TEM image of rat gastric glands recorded at a direct magnification of $\times 3,500$. Distributions of chief cells of gastric glands are clearly visible. A large amount of zymogen granules in the main cell are also seen at high contrast.

Fig. 5 shows a digital TEM image of the main cell of rat gastric glands at a direct magnification of $\times 15,000$. Zymogen granules are clearly seen and microvilli are also seen in their lumen. Black particles (presumably colloidal gold) are seen in a high electron density area which is close to a part of zymogen granules and lumen of gastric glands.



File Name = 03S.tif
PLA2
TEM Mode = HC-ZOOM

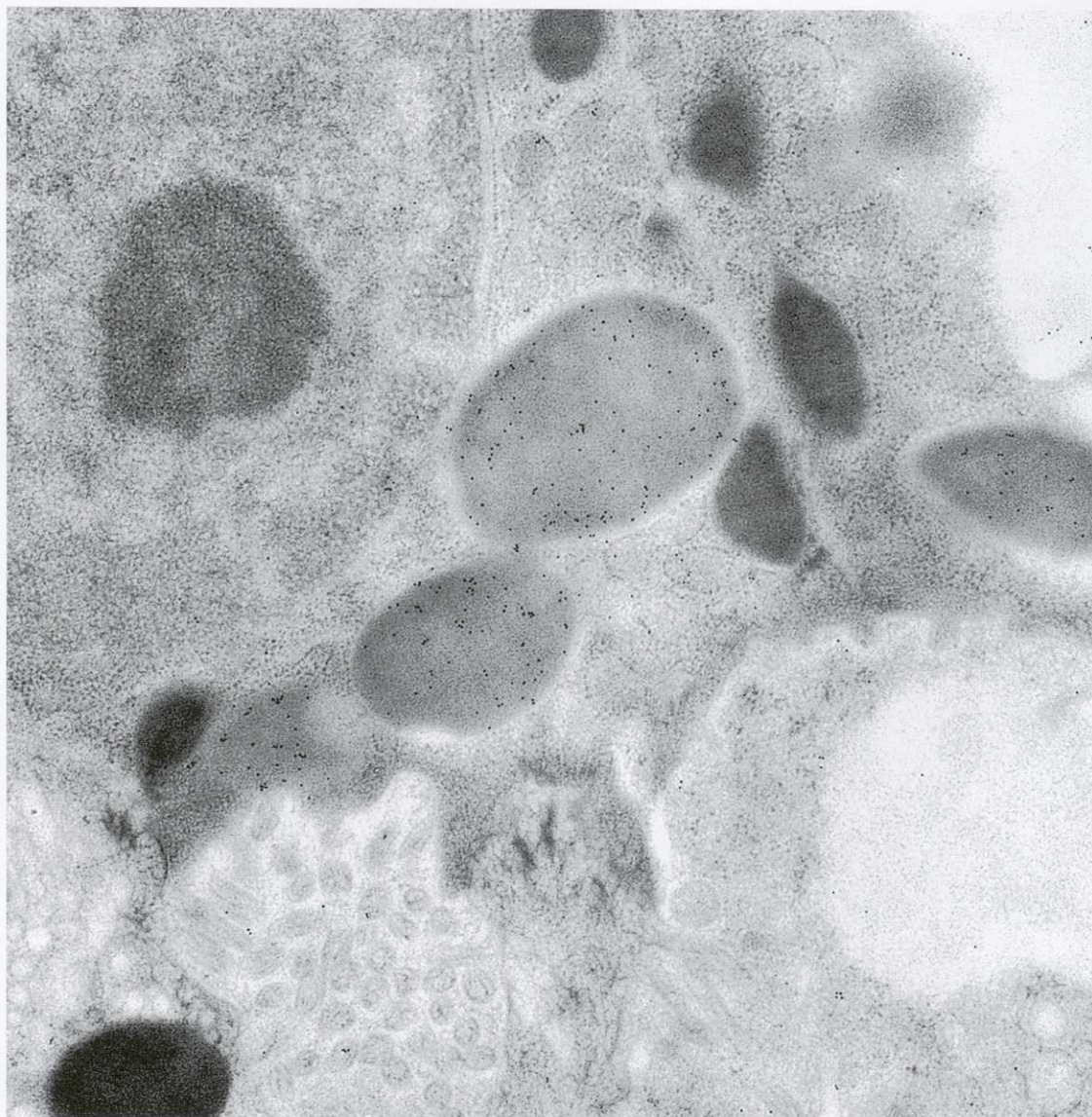
500 nm
HV = 100 kV
TEM Mag = 15000 \times
X = 144.9 Y = -18.5
H-7600

Fig. 5 Applications of digital TEM images —3—

Sample: Rat stomach, Acc. volt.: 100 kV, Direct magnification: $\times 15,000$

Fig. 6 shows the same area of interest at a higher magnification. We can see a number of colloidal gold particles on zymogen granules. We can also see some distributions of colloidal gold particles in a high electron density area near gastric glands. These are not distributed uniformly but rather locally. A relatively high electron density area where we can see colloidal gold par-

ticles is very close to the lumen of gastric glands and a part of the area is deformed. As we can see some colloidal gold particles in the lumen of gastric glands, we assume that some exocytosed zymogenic contents are about to be released¹⁾. These are all digital images but they are quite useful for observation of fine structures.



File Name = 04S.tif
PLA2
TEM Mode = HC-ZOOM

500 nm
HV = 100 kV
TEM Mag = 30000 ×
X = 144.2 Y = -18.3
H-7600

Fig. 4 Applications of digital TEM images —4—

Sample: Rat stomach, Acc. volt.: 100 kV, Direct magnification: × 30,000

5. CLOSING REMARKS

We have introduced an image database, network, and quality digital TEM images using immunological microscopy specimens. Coupled with the wide-spread use of IT and development of associated infrastructures, genome analysis and other research projects are progressing at a very high speed. Electron microscopy is not an exception. Quick image acquisition will be very important for timely results in the future. The H-7600 allows networking with TV camera and other peripheral equipment for database which may be shared by a number of users. We trust that this system capability will be useful in the coming years. We wish to thank Prof. T. Suganuma, Department of Anatomy, Miyazaki Medical College for providing specimens.

REFERENCE

- 1) Reappraisal of potassium permanganate oxidation applied to lowicryl K4M embedded tissues processed by high pressure freezing /freeze substitution, with special reference to differential staining of the zymogen granules of rat gastric chief cells. Sawaguchi, A., et al., Arch. Histol. Cytol., 447-458 (1999)