Teaching biology through remote access microscopy

G. Nagy, G. Pinczes, P. Papai, G. Kiraly and G. Banfalvi

Department of Microbial Biotechnology and Cell Biology, University of Debrecen, 4010 Debrecen Hungary

The aim of this chapter is to introduce remote access microscopy in biology teaching, trough i) electron microscopy and ii) time lapse microscopy. i) Our old TESLA BS 540 transmission electron microscope was converted from analog to digital processing mode (dTEM). The conversion serves as a tool to teach electron microscopy in student practicals. dTEM also provides information through remote access to highschool students in the region, whoo are not present, but occasionally visit the university and have internet access. ii) The other remote access facility is time-lapse microscopy designed to follow the movement of individual cells from distant locations. Time-lapse microscopy can follow cell growth for an unlimited period of time under physiological conditions without opening the CO_2 incubator. This technical development provides postgraduate, undergraduate and high school students remote acces to control cell growth on a daily routine basis. Due to protective instructions that have been incorporated in the software the microscopic techniques: a) became easier to use, b) are time and cost-effective, c) igital micrographs provide a wealth of analytical information, d) the research data are of publication quality, e) permit real time photo sharing, f) data can be analysed and evaluated by independent users at different locations

Keywords: digital camera; remote control; image acquisition; low-light imaging; cell morphology; cellular movement

1. Introduction

1.1. Theoretical background

The higher resolution of transmission electron microscopes (TEMs) than that of light microscopes is due to the small wavelength of electrons transmitted through a thin specimen. The image formed from the interaction is magnified and focused on a screen, film or CCD camera. Although, the magnification of TEM can go up to 750,000, it is not the magnification that matters. The critical factor is resolution that can be at best about 1 nm (objects smaller than 1 nm cannot be distinguished) and provides about 100 times better resolution than light microscopy. Our TESLA BS 540 TEM allows the examination of fine structural details to a resolution of 3 nm. In biological studies very thin sections of tissues can be observed at magnifications of about 120,000 times.

The drawbacks of the TEM technique are: a) the time consuming preparation of samples thin enough to be electron transparent, b) the relatively small field of view questioning the characteristic representation of the sample, c) the damaging effect of the electron beam especially in biological samples. The damage is due to the release of electrons by heating a very thin tungsten filament in a vacuum. The released electrons are then submitted to a voltage difference between 60-120 kV between the cathode and anode. The accelerated electrons passing through the central hole of the metallic plate anode and the electromagnetic lens generated by the condenser coil form a constant beam penetrating the tube of the microscope and the specimen. The magnified image is projected through further magnifying coils. The fluorescent screen image can be seen directly by the operator and photographed with a camera or CCD detectors [1]. Two basic types for transferring photons to the CCD have been developed. In lense–coupling the post-magnification can be changed, but due to the poor efficiency (~0.5%) the detection of single electrons is impossible. Fiber-optic coupling provides a much higher efficiency (~ 60%).

The capture of cellular movement through the microscope by time-lapse photography and turning it to video microscopy movies also referred to as cinemicrography [2], is an efficient teaching tool in cell biology practicals. The introduction of analog video-capture technology in the 1980s expanded the use of light microscopy as an analytical tool [3,4]. This technique has been replaced by computer-based digital image-capture systems [5,6]. The ease of use, low-noise, high quantum efficiency of digital micrography systems enabled the study of dynamic events in cell biology. Further development was the establishment of time-lapse video-microscopy to visualize longer periods of dynamic processes of morphological changes [7,8] such as the rapid movement of apoptotic cells referred to as „dance of death" [9,10]. The major drawback of time-lapse microscopy remained that cells growth could be followed only for a limited period of time up to 96 h [7] outside CO_2 incubators under non-physiological conditions.

To overcome technical hindrances that have prevented our biology students (yearly 150-200) from electronmicroscopy and video microscopy practicals students, we have converted our TEM from analog to digital mode and with the help of students we have developed our time-lapse microscopy system to visualize cellular motion and morphological changes of individual cells for an unlimited period of time inside the CO_2 incubator. As a result in the practicals all of our biology students can now see the attachment of cells, monolayer formation, movement of cells in suspension culture, cell growth from low density to high confluence, observe cellular vibration and movement,

detachment and rounding up of dividing cells, loss of contact with neighbouring cells, distinguish between patterns of apoptotic and necrotic cell death.

2. Research methods and tools

2.1. Digital conversion of analog signals to digital data

The transmission electron microscopy (TEM) diffraction patterns from thin sections of tissues were measured at the room temperature T_0 with our TESLA BS-540 microscope operating at 80 kV with λ = 0.00418 nm. Electric screwdriver (Einhell, Hungaria kft., Budapest) were coupled to the computer controlled electric power steering stage control unit that allows the precise identification and return to the same position of specimens on the stage plate. The digital conversion of the TESLA BS 540 transmission electron microscope involved: a) electric control rather than manual stage movement, b) application of digitazing device, and c) Custom-built camera with Sony Exview-HAD CCD sensor.

2.2. Cell growth

HaCaT human skin cell line consisting of keratinocytes mimic many of the properties of epidermal keratinocytes and can differentiate under appropriate conditions [11]. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM-F12, also known as Ham's medium) (pH 7.2, Gibco BRL, Gaithersburg, MD) Sigma, St. Louis, MO), pH 7.2, in the presence of 5% fetal bovine serum and penicillin/streptomycin. The monolayer cell culture was grown in T-flasks in a CO_2 incubator at 37°C and checked for adherence after 24 h.

Human erythroleukemia K562 suspension culture was grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). This cell line was established from the pleural effusion of a 53-year-old woman with chronic myelogenous leukemia [12].

2.3.Time-lapse photography

Our custom built system consists of four inverse microscopes located inside a Sanyo MCO-18AIC CO_2 incubator each equipped with a high sensitivity video camera connected to a dual image acquisition computer system [13]. Custom-built illumination was developed to minimize heat- and foto-toxicity. Operation of the spectrally warm-white light emitting diodes were synchronized with image acquisition periods. Exposure times were minimized to avoid phototoxicity. Cell cultures in T flasks were placed on inverse microscopes. Transmission light microscopic images of cells were taken every minute. Image acquisition parameters were tuned for maximal greyscale dinamic range resolution averaging 5 auto-intensity histogram equalized images. The time difference between images taken from each flask was not more than 6 seconds ± 8%. The high resolution screen of the computer was divided into four portions showing side-by-side the morphological changes of the control and three treated cells cultures. The time of exposure was indicated at the bottom of each frame. Exposures were converted to videofilms by speeding up the projection to 30 exposures/s. Individual cells of monolayer cultures were selected for analysis. Individual photographs were chosen as panels shown in the figures. Time-lapse photography of individual cells was used to visualize the growth profile of individual cells allowing distinction among normal and irregular cell divisions.

3. Results

3.1. Conversion of analog TEM signals to digital data

The transmission electron microscopy (TEM) diffraction patterns from thin sections of tissues were measured at the room temperature T_0 with our TESLA BS-540 microscope operating at 80 kV with λ = 0.00418 nm. Electric screwdriver controlled electric power steering stage control unit and allowed the precise identification and return to the same position of specimens on the stage plate. The converted and computer directed TESLA BS-540 microscope is shown in Fig. 1. The electric screwdrivers are indicated with the upper two circles in Fig. 1A.

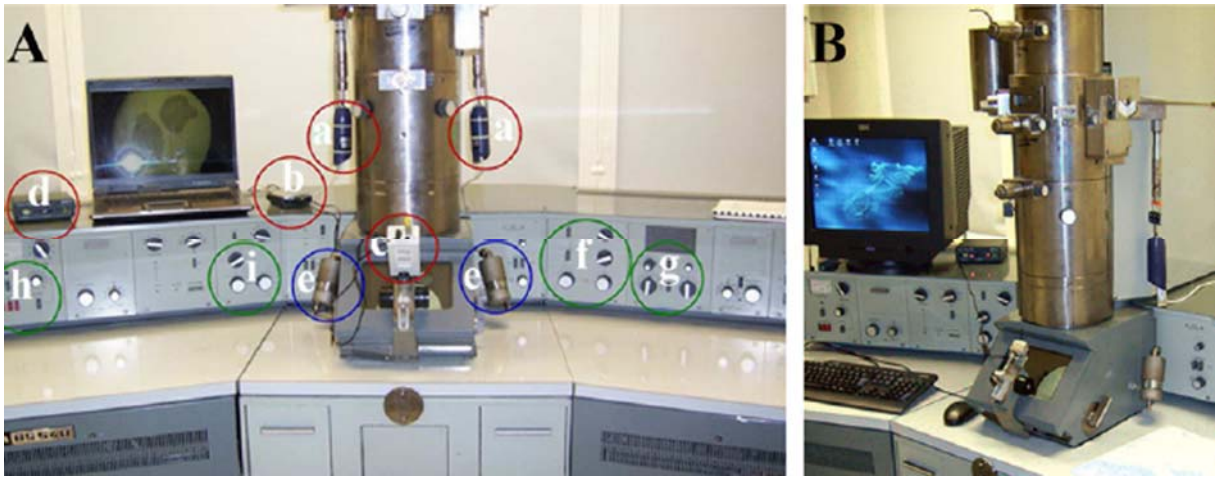

Figure 1. Conversion of TESLA BS 540 from analog to digital operation.A) a,b,c,d indicate new devices (upper circles), e) shows omitted devices (middle two circles), and f-i are unchanged functions (left and right circles). B) Visualization of chromosome condensation by dTEM using TESLA BS 540 microscope.

The digital conversion of the TESLA BS 540 transmission electron microscope involved:
a) electric control rather than mechanical stage movement,
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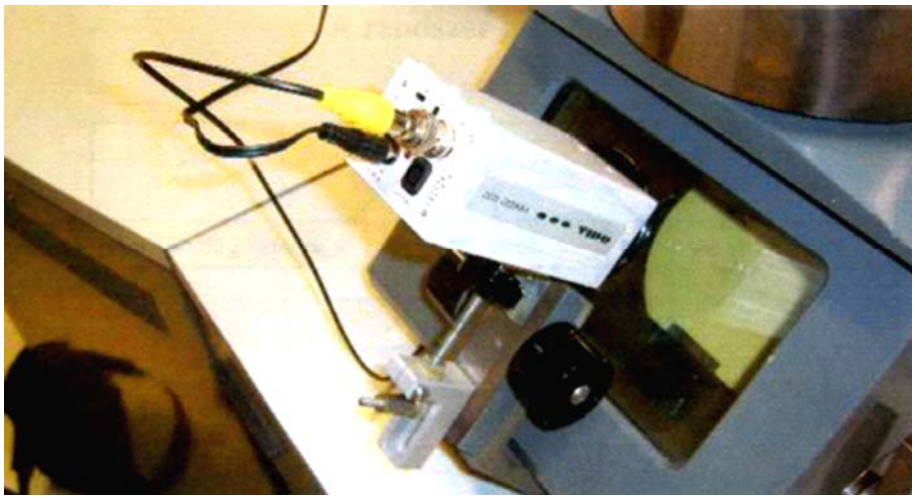

Figure 2. Attachment of video camera to the fluorescent screen of the TESLA BS 540 microscope. The mounting of the camera was done by using the leg-support of a stereomicroscope. This solution allows the movement of the camera to left and right and maintain the original mechanics with the original visual function of the microscope.

Among the new devices are electronic motors (electronic scew drivers) that move the specimen table (Fig. 1a), the digitizing device (Fig. 1b), CCD camera (Fig. 1c), and the engine steering unit (Fig. 1d). The old manual manipulators (Fig. 1e) have been switched off, but can be swithched back anytime. Other operating functions such as electron focusing (Fig. 1 f), switch of magnification (Fig. 2g), filament (Fig. 1 h) and condensor adjustments (Fig. 1 i) remained unchanged. The whole operation system is demonstrated by the visualization of condensing chromosomes subjected to chromatin image analysis (CIA), a software developed in our laboratory (Fig. 1B). A closer view of the attachment of the CCD camera to the window of the TESLA BS 540 dTEM is shown in Fig. 2.

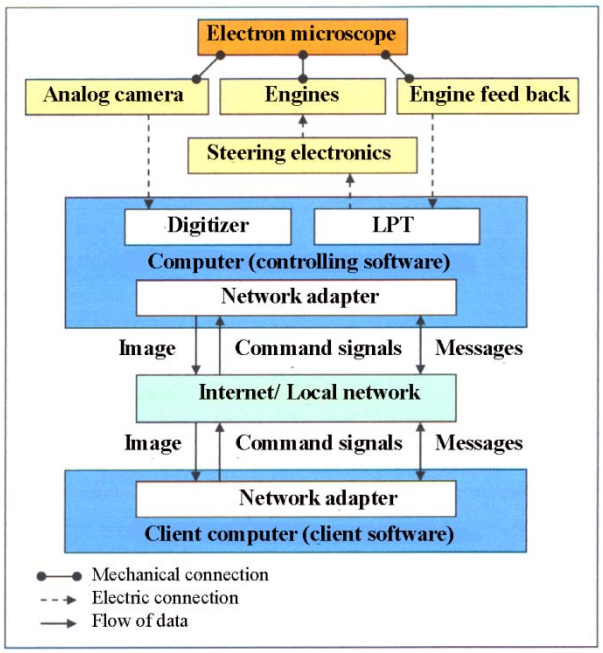

Figure 3. Connection diagram of the operation unit of dTEM. The images of the specimens of the converted TESLA BS 540 microscope are transmitted by a video camera, digitalized and transmitted by the server computer. Servos and feedback controllers are connected to a custom built controller box.

3.2. Structure of the new internet tele-electronmicroscopy (iTEEM) system

The main function of the server software is to send the information gathered by the camera from the microscope through the Internet to the client computers, while local control of the microscope is also posible. The server application is able to send and receive text messages with the client computers, opening a chat-like channel between the operator and users. Frequently used command phrases related to the local control of the microscope are also available as keystroke shortcuts for convenient use. The local operator can grant the right of control to any client. Supervision of less experienced remote users serves to protect the sample. While controling the electron microscope remotely, desynchronisation between the video stream and control keystrokes may occur. The lagging depends on several parameters. The first and most significant source of such shift is the communication time between the two computers, known as „ping-time" that could not be affected, since it is a physical parameter of the network between the computers. The second parameter is the time of the analog/digital conversion of the video signal *via* the hardware. By optimalizing the control system this frame-lapse could be reduced to 100ms. The third reason of time-lapse is image enhancement. Using high magnification (up to x120,000) drastically affects signal/noise ratio caused by the low light conditions on the phosphorescent screen of the dTEM. This effect can be compensated by built in algorithms such as averaging of frames, auto-intensity or display transfer method. Averaging of frames can reduce frame-rate up to 3 frame/sec, giving some difficulty in remote control. To improve the overall performance of the system several changes has been made. Converting the 720x576 pixel frames to single channel-8-bit grayscale reduced the necesarry bandwidth to 40%. We have also incoroprated a so called „quick-mode" by using image compression and reduced image enhancement with remote user cruising around the specimen. When the region of interest is selected, the software switches back to uncompressed, enhanced mode of operation. The schematic view of the directing and client softwares are summarized (Figure 3).

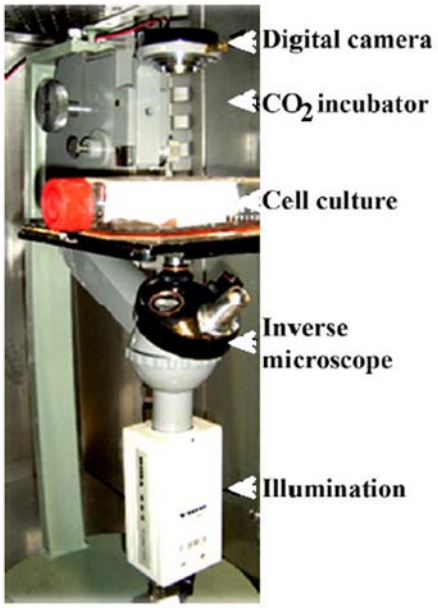

Fig. 4. Long-term scanning system. One of the two identical inverse microscopes one is shown. Scanning systems were constructed and placed in CO_2 incubator. Each digital scanning system consists of a digital camera, cell culture in T flask sitting on inverse microscope illuminated with a white light emitting diode. Long-term scanning microscopy shows the apoptotic effect 24 h after 100 μM $NiCl_2$ treatment of K562 cells (see videofilm in the attachment).

3.3. Long-term imaging of cell growth and morphological changes

We have imaged continuously HaCaT cells under normal, apoptotic and necrotic conditions (see Supplemental Movie 1) to show biology students characteristic morphological changes upon genotoxicity.

Time-lapse video microscopy has been developed to visualize morphological changes in cell cultures [7,8,14]. As these studies took place outside CO_2 incubators, they were not carried out under physiological conditions. Consequently, cells could be kept alive only for a few days [7]. We have improved this technique, by placing the video microscope in a CO_2 microscope and replacing cell culture medium by a peristaltic pump (Fig. 4). This allowed us to see morphological processes of cell growth and cell death (apoptosis, necrosis) for a longer period of time (1 week) (Fig. 5).

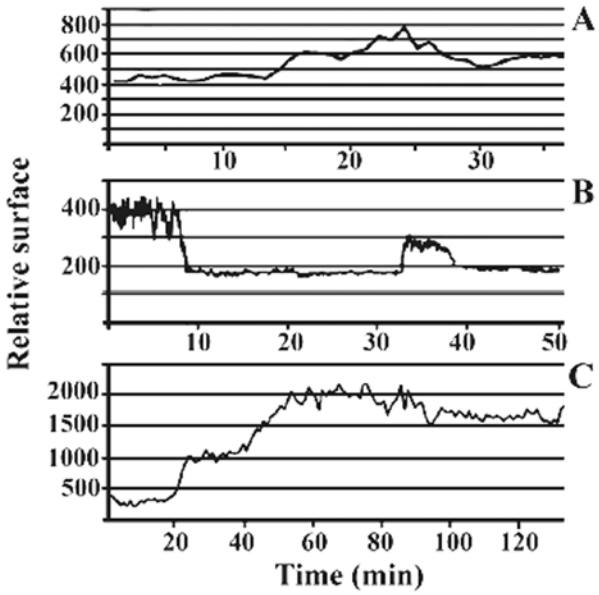

Figure 5. Graphic presentation of changes in visible cell surface using video microscopy. a) Profil of HaCaT cell growth and division under normal conditions. b) Apoptotic reduction of cell surface upon 1μM $Pb(NO_3)_2$ treatment. c) Necrotic cell surface increase induced by 50 μM $Pb(NO_3)_2$. Modified with permission of Nagy et al. [13].

4. Discussion

The transformation of the analog TESLA BS 540 transmission electronmicroscope to digital mode has been combined with remote operation by clients who have been provided free access to the system. This dTEM became an essential tool in teaching electron microscopy in biology practicals both by physically operating the instrument locally and from distant classroom locations. Recently researchers, PhD students, postgraduate and undergraduate students have access to the remotely controlled electron microscope on a daily routin basis. The images of digital electromicrographs provide a wealth of analytical information regarding individual organels that can be studied by students in a laboratory course, equipped with basic computer tools. The conversion of our TESLA BS 540 from analog to digital mode resulted in: a) digital images that portray the true nature of cellular constituents, b) made it easier for students to grasp the function of cells and their constituents, c) beside researchers, students can help to make high resolution micrographs, d) data of micrographs can be stored in computer for further analysis, e) handling of the system became easy and safe due to the inbuilt electronic security program. The images of individual organels are not just pretty pictures; digital electromicrographs provide a wealth of analytical information that can be readily used by students in a laboratory course with basic computer tools. Each student or group of students can be given the image file(s) and use inexpensive/free software to conduct very informative image analysis experiments. Such analytical software tools have been developed by the Laboratory for Optical and Computational Instrumentation, LOCI, at the University of Debrecen.

Another important development in biology teaching was the construction of time lapse video microscopy system to follow the movement and morphology of individual cells under physiological and genotoxic conditions. The system consists of four inverted microscopes located in the CO_2 incubator connected to charge-coupled device cameras and computers. Photographs taken every minute were converted to a video sequence. An example of how the movement of K562 cells is changing upon 100 μM $NiCl_2$ treatment is shown in the attachment (internet site will be provided). The system was also applied to describe cell surface changes after the genotoxic (apoptotoc, necrotic) treatment of HaCaT cells.

To summarize our educational progress we have converted our analog electronmicroscope to digital mode and have applied video microscopy in cell biology practicals. Beside the visualization of cellular movements and morphological changes exerting a strong motivation effect on students and researchers, technical changes contributed to studies related to chromatin condensation [15].

5. References

[1] Gruner SM, Tate MW, Eikenberry EF. Charge-coupled device area X-ray detectors. Review of Scientific Instruments, 2002;73:2815–2842.
[2] Rose GG. Cinemicrography in Cell Biology. New York: Academic Press; 1963.
[3] *Inoué S.* Video Microscopy. *New York: Plenum Press,* 1986.
[4] Salmon ED. VE-DIC light microscopy and the discovery of kinesin. Trends in Cell Biology, 1995;5:154-158.
[5] *Inoué S, Spring K.* Video Microscopy*, 2nd ed. New York: Plenum Press;* 1997.
[6] Sluder G, Wolf D. Digital microscopy: a second edition of video microscopy. Methods in Cell Biology, 2003;72:1-523.
[7] Pulkkinen JO, Joensuu LH, Martikainen P, Servomaa K, Grénman R. Paclitaxel-induced apoptotic changes followed by time-lapse video microscopy in cell lines established from head and neck cancer. Journal of Cancer Research and Clinical Oncology,1996;122:214-218.
[8] Rieger MA, Schroeder T. Exploring hematopoiesis at single cell resolution. Cells, Tissues and Organs, 2008;188,139-149.
[9] Bernard J, Malawista SE. Remebrance of Professor Marcel Bessis (1917-1994). Blood Cells, Molecules and Diseases, 1995;21:152-155.
[10] Humke EW. Web Alert. Apoptosis. *Chemical Biology,* 2000;7:R48-R49.
[11] Boukamp P, Petrusevka RT, Breitkreut D, Hornung J, Markham A, Fusening NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte line. Journal of Cell Biology, 1988;106:761-771.
[12] Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood, 1975;45:321-334.
[13] Nagy G, Pinter G, Kohut G, Adam A, Trencsenyi G, Hornok L, Banfalvi G. Time-lapse analysis of cell death in mammalian and fungal cells. DNA and Cell Biology, 2010;29:249-259.
[14] Forrester HB, Albright N, Ling CC, Dewey WC. Computerized video time-lapse analysis of apoptosis of REC:Myc cells X irradiated in different phases of the cell cycle. Radiation Research, 2000;154:625-639.
[15] Banfalvi G. Apoptotic chromatin changes. Springer Science + Business Media B.V., Dordrecht, New York, London, 2009.

