ARTICLE

Creating a Histology–Embryology Free Digital Image Database Using High-End Microscopy and Computer Techniques for On-Line Biomedical Education

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The development of new technology and the possibility of fast information delivery by either Internet or Intranet connections are changing education. Microanatomy education depends basically on the correct interpretation of microscopy images by students. Modern microscopes coupled to computers enable the presentation of these images in a digital form by creating image databases. However, the access to this new technology is restricted entirely to those living in cities and towns with an Information Technology (IT) infrastructure. This study describes the creation of a free Internet histology database composed by high-quality images and also presents an inexpensive way to supply it to a greater number of students through Internet/Intranet connections. By using state-of-the-art scientific instruments, we developed a Web page (http://www2.uerj.br/~micron/atlas/atlasenglish/index.htm) that, in association with a multimedia microscopy laboratory, intends to help in the reduction of the IT educational gap between developed and underdeveloped regions. ***Anat Rec (Part B: New Anat) 273B:126–131, 2003.***

KEY WORDS: database; video microscopy; digital imaging; microscopy; medical education; virtual microscope; computer-assisted learning; CAL

INTRODUCTION

The understanding of histology and embryology is a process that starts with the correct interpretation of microscopy images, which leads to a better comprehension of morphology and the morphogenesis of cells, tissues, and organs. However, until recently, the access to this visual information by students was limited to periodic and short practical microscopy sessions backed up by textbooks and printed atlases. The limited number of microscopes in the medical and biological institutions of underdeveloped areas was an added problem. Moreover, in most universities, before the practical classes of histology and embryology, the histological preparations were presented by the professor with a micro-slide projector. Because of poor resolution and illumination, the projected images were delivered with poor quality, hampering the correct visualization of the samples by the students.

In the 1980s, the advent of video-microscopy revolutionized histology classes by remarkably improving the image quality (Inoué and Spring, 1997). Nevertheless, despite the above-described advances, some difficulties still remain: the costs of video-microscopy systems are usually prohibitive for many schools in underdeveloped countries and the restricted number of light microscopes consequently limits the access of the students to a greater quantity of visual information.

The availability of the Internet and of advancements in computers coupled with microscopes created untold possibilities for improving histology, embryology, and anatomy courses (Trelease et al., 2000; Heidger et al., 2002). These advances will probably help to close the educational gap between developed areas and those that are in the process of catching up, by making robust information technology (IT) infrastructure and opportunities more available. In addition, there is the possibility of direct access to light and electron microscope images through the creation of image databases and the transmission of such archives to several sites by Intranet and Internet. This access allows students from different institutions or those with a limited number of micro-

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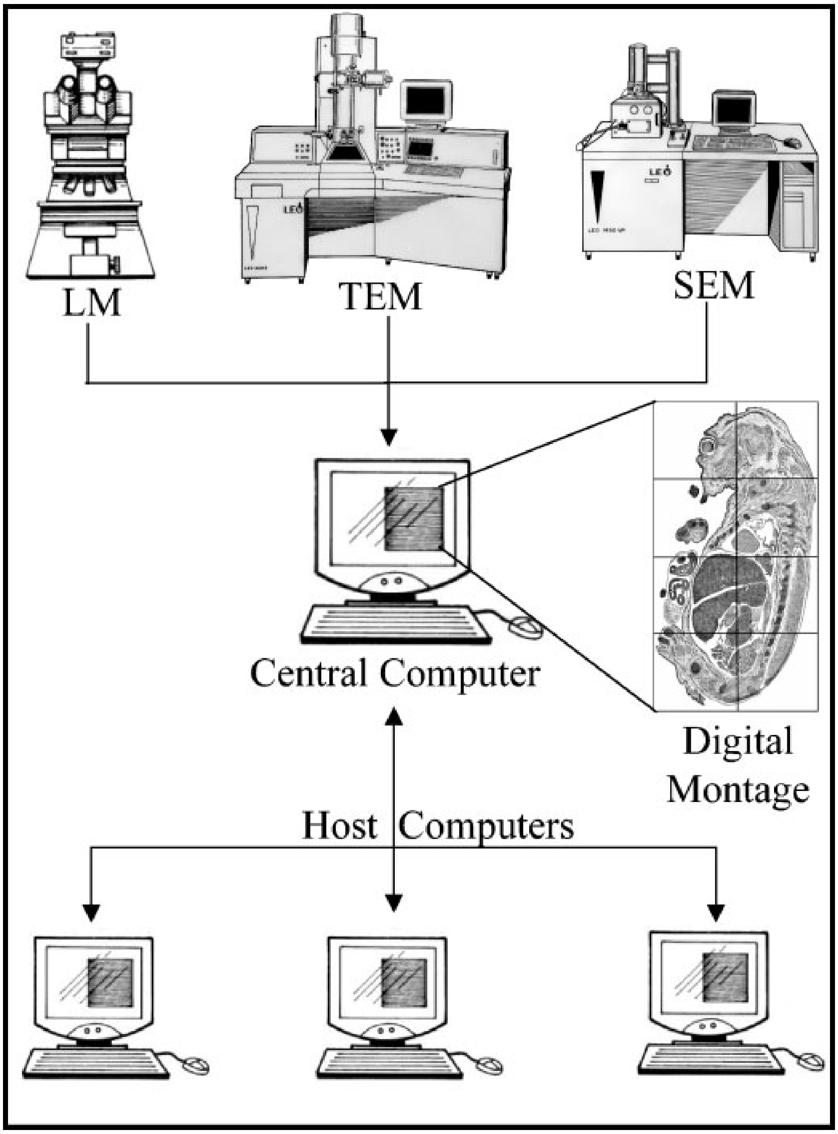

Figure 1. Schematic representation of the major steps concerning the construction of the histology database. After the acquisition of high-resolution images in the digital microscopes (LM, light microscope; TEM, transmission electron microscope; SEM, scanning electron microscope), the frames are transferred to the central computer (central computer) and a digital image montage (digital montage) is created. The computer is used to create the database and is connected to other computers (host computers) by Internet or Intranet.

scopes or unable to acquire a sophisticated video-microscope system to easily download images developed by a state-of-the-art equipped center. Also, the unlimited and plastic virtual space, contrasted with the greatly limited, expensive, and fixed space available for pictures in books, introduces a vast amount of information to the students (Brinkley et al., 1997).

However, a new step for microanatomy could take place by bringing together the new techniques and protocols of image processing for the production of single image montages. By using the high-resolution frames obtained by wide-aperture light microscope lenses and the high-quality magnified electron microscope images, it is possible to create montages showing larger microscopic fields of view with greater resolution (Monteiro-Leal et al., 2003; Romer et al., 2003).

This study sets out how to develop and assemble a high-quality histology database to be used by the students and by creating a multimedia laboratory, with the association of computer, Intranet or Internet, microscopes, and television, a less-expensive way to present the information. The impact of this work on the Brazilian and international communities are also reported.

METHODOLOGY

Preparation of the Histological Sections

Healthy Wistar rats were killed in a CO_2 camera (following the directions set out by the Ethics Committee for use and care of experimental animals from the Biological Institute of the State University of Rio de Janeiro).

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Their organs and tissues were extracted, quickly finely sectioned, washed in saline buffer, and then prepared by following the specific protocols for light and electron microscopy, as described before (Karnovsky, 1965; Stoward and Pearse, 1980; Bancroft and Stevens, 1996; Monteiro-Leal et al., 1996; Bozzola and Russell, 1999).

Digital Microscopes

The three microscopes (light, scanning, and transmission electron microscope) were connected to and controlled by computers. The images visualized in the light microscopes, using high numerical aperture and aberration-free objectives, were after-

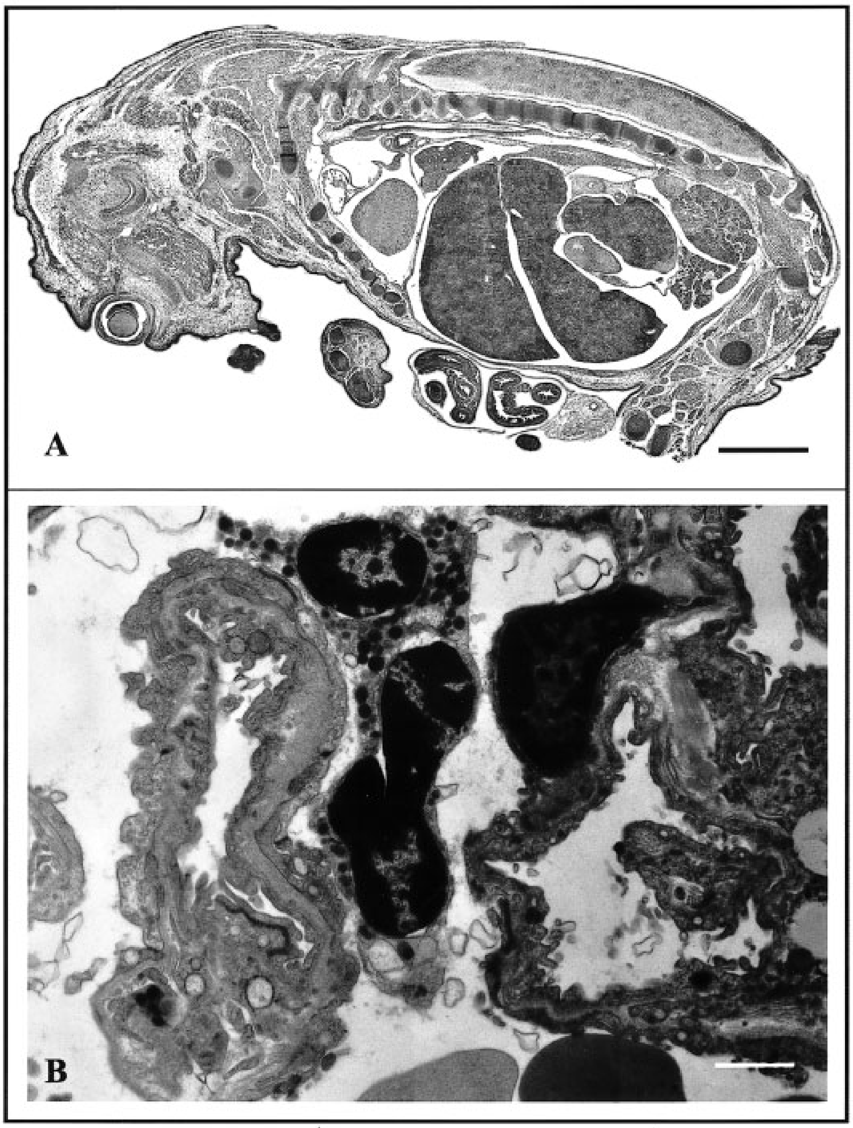

Figure 2. Digital montages created by assembling several high-resolution microscope images. **A:** The result of the montage of 200 single high-resolution, small-field images in a final high-resolution, large-field frame. This picture shows a 14-day-old mouse embryo. **B:** The same procedure, this time performed with electron transmission microscopy frames. The picture shows a high-resolution, large-field montage of a lung section obtained after the computer association of 16 single frames. Scale bars = 1 mm in A, 3.5 μm in B. (Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.)

ward acquired with the help of a digital camera (SoundVision SV-Micro). The pictures obtained by using the scanning electron microscope were digitized in the machine itself, and the ones observed in the transmission electron microscope were acquired with the associated slow-scan cooled CCD camera (Proscan elektronische Systeme, Scheuring, Germany) on axis camera (Hiller et al., 2000; Campanati et al., 2002; Monteiro-Leal et al., 2003).

Central Computer

To process, analyze, and perform the image montages, a central computer was assembled and the KS 400 (Zeiss-Vision, Jena) and the SIS-Auto (SIS-SoftImage, Münster) image processing systems were installed. This computer was a Pentium III, with 512 MB RAM memory, and 20 GB disk space connected to a network working at 2 MB per second.

Software

The Web site was created by using the basic HTML-creator software that is part of Netscape software (Netscape Composer). The images were further processed and compressed with Paint Shop Pro 6.0 (JASC Software, Inc., USA. 1999), and some montages were created with the use of the MIA – module from SIS-Auto and the Ipdeluxe image processing software (Wootton et al., 1995; Monteiro-Leal et al., 2003).

ACQUISITION, MONTAGE, AND PRESENTATION OF DIGITAL IMAGES

To obtain high-quality digital images, our microscopes were connected to a central computer that stored all images transferred by Intranet (Figure 1). The central computer (Central Computer, in Figure 1) hosted the database and the *mother* Web site, a mirror of the published Web site, located in the main computer of the University. The images produced by the three microscopes were improved and compressed using the specific software (described in the Methodology section) in the central computer. Digital image montages were prepared (Digital Montage in Figure 1) to prevent poor resolution associated with images acquired with enlarged field of view, using, for example, low-magnification light microscope objectives; and the acquisition process by digital camera, in the case of the transmission electron microscope (Inoué and Spring, 1997; Campanati et al., 2002; Monteiro-Leal et al., 2003). By the use of high numerical aperture objectives in light microscopy or by the use of high magnification with the transmission electron microscope, high-quality images were prepared that, on the other hand, negatively present a smaller field of view. The montage of several sequential acquired images (digital montage in Figure 1) by the computer solved the above-mentioned problem about the limited field of view of these images, resulting in high-quality frames with enlarged field of view (Figure 2A,B; Monteiro-Leal et al., 2003). Afterward, the images were then transferred by Internet or Intranet to the host computers (host computers in Figure 1).

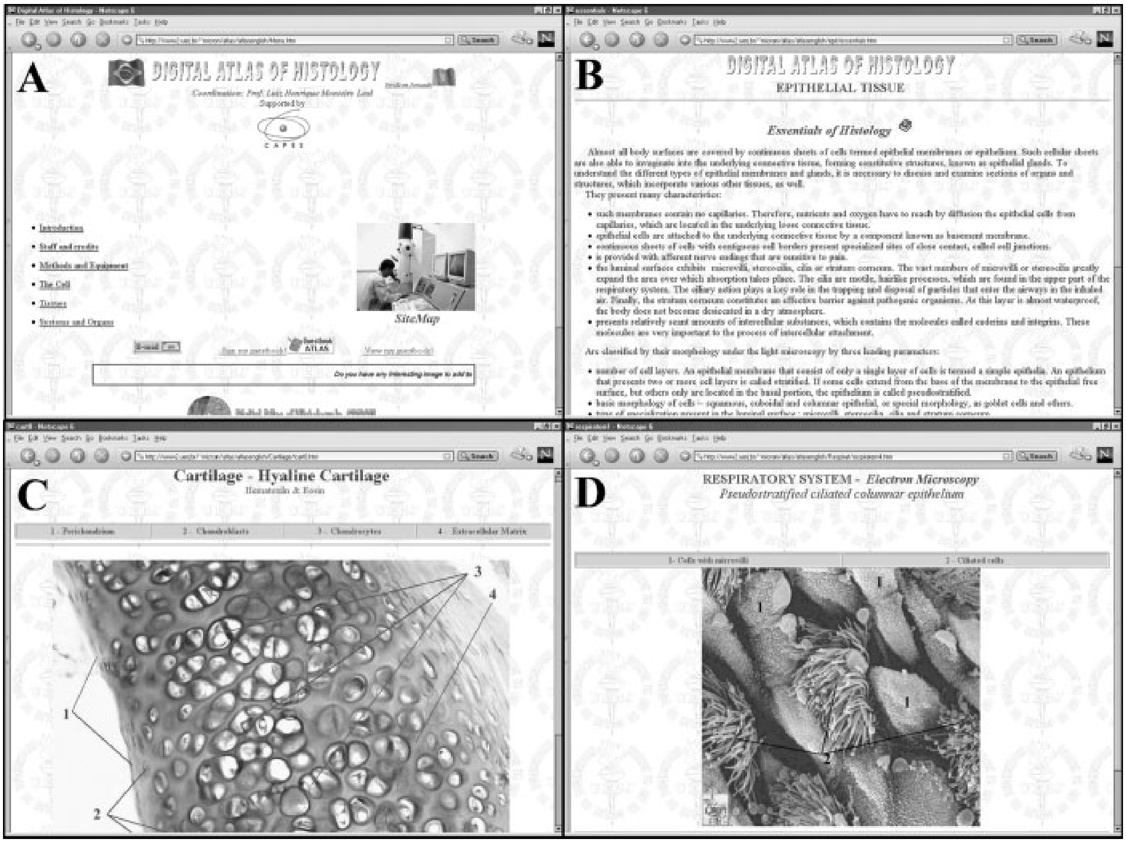

Figure 3. Sample images obtained from the Digital Histology Atlas. **A:** The menu page from where the other 350 English pages can be opened from the Atlas. **B:** An example of the short explanatory texts related to the images. **C:** A light microscope montage that opens one tissue image database. **D:** One sample from the several scanning electron microscope images that can be found in the Atlas. (Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.)

IMAGE COMPRESSION AND PREPARATION OF THE WEB SITE

When image montages were created, their higher quality had the drawback of producing huge archives; some files greater than 80 MB (TIFF files). These archives were too heavy to be transferred between computers by Internet. To solve this problem the images were compressed into JPEG files using the software listed in the Methodology section. The initial high resolution of the images, as described above, compensates for the partial loss of resolution caused by the compression process.

Afterward, the images were uploaded in the Web site using Netscape Composer and linked serially to a principal page that shows the options menu. The access to the Web site (http://www2.uerj.br/~micron/atlas/atlasenglish/index.htm), called Digital Atlas of Histology (Figure 3A–D), was free. First-time users were requested to fill out an electronic questionnaire, which was afterward used to collect information regarding the users. The Atlas was prepared in Portuguese and English, and accompanying each image was explanatory text. It was launched on-line in 1999 and since 2001, a new-access counter showed that there have been more than 35,000 visitors, an average of 3,000 to 4,000 guests per month.

DIGITAL ATLAS USER PROFILE

The profile of the visitors to the Digital Atlas, obtained by the on-line questionnaire (10,500 questionnaires), is analyzed in Figure 4. Part A displays the percentage of regional access in Brazil, which as expected, represents a very high degree of access (90%). It shows that users in the Southeast region performed more than 50% of the accesses and, together with the Southern region, represented 70% of all accesses in the country. These regions are the most developed in Brazil. Part B shows the same analyses undertaken in A, but refers to “international access.” From Europe came 39% of the accesses, followed by North America with 25% (USA corresponded to 70% of the accesses) and South-America with 22% (the Brazilian accesses were not computed). Guests from Asia correspond to only 7% of the accesses. Part C presents an analysis of the above-mentioned data concerning users’ educational status or background. The data shows that 52% of the guests are university students, followed by graduates and high-school students.

MULTIMEDIA LABORATORY

The digital histology database was either accessed at home individually or through computers in libraries or in any other public place (data not shown). Moreover, the image databank was used during the normal practical classes in the microscope laboratory. To make these new technologies available to all students and to improve the content of the lectures, the following methodology was intro-

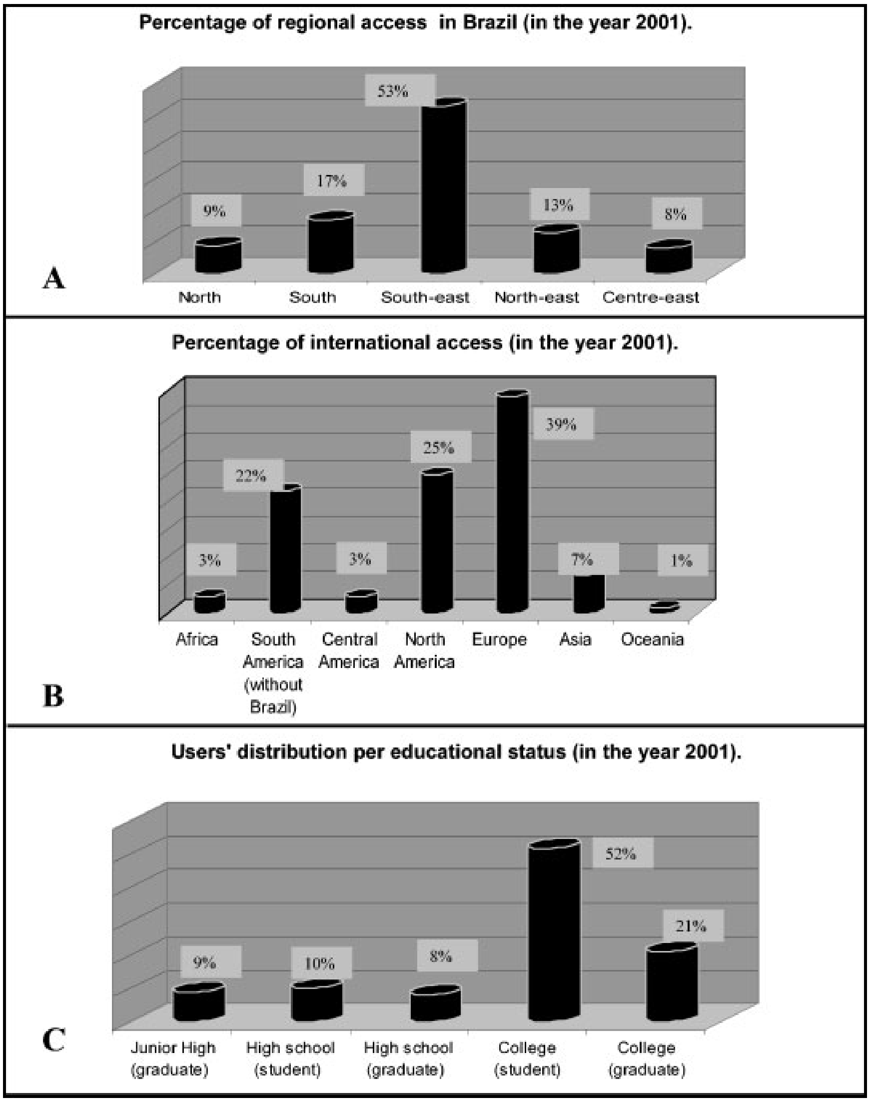

Figure 4. Graphic distribution of the Digital Histology Atlas. **A:** The percentage of access in 2001, separated by regions in Brazil, shows that the great majority of guests (70%) came from the South and Southeast, which are the most-developed regions of the country. Students from Brazil represent 90% of all accesses. **B:** The same analysis as in A but refers to the distribution of international visitors. Europe has the greatest access majority, followed by North America (the students from the United States represent 70% of all accesses) and South America (the guests who accessed from Brazil are not included). **C:** Profiles (educational background) of the guests who accessed the Atlas during the past year. The data show that the great majority of visitors were college students.

duced in the practical sessions. Besides the video-microscopy used by the professors at the beginning of the classes to show what preparations would be taught on a specific day, the day's theme could be also reiterated by the use of the high-quality images obtained by light and electron microscope and stored in the Digital Atlas. To do this, a computer was connected by Intranet with the central computer (where the databank is situated), and by using a switch, the images of the Digital Atlas were presented on the same television monitor, which is also connected to the video-microscope (the same one that had been previously used to show the day's preparations). The computer was equipped with a fast Internet connection and with a video board with analog output. A less costly design was developed for a small educational unit in northern Brazil, where a computer with Internet was connected to a 34-inch TV. It was possible, with the help of such a system, to use the Digital Atlas through the Internet instead of the more expensive video-microscopy equipment, also excluding the necessity for histological slides. Alternatively, some modern televisions are equipped with a specific input for directly plugging into a computer, functioning as a computer monitor. In such cases, one can use any regular computer, avoiding the use of a video board with analog output.

FINAL CONSIDERATIONS

Making Internet access and computer technologies available to an increasing number of students is a great challenge for the future. The opportunity to use these advances in education, in particular in biomedical education, should be a priority. Some published papers are concerned about the impact of the new technologies on education (Brinkley et al., 1997; Clark, 2001; Lin and Hsieh, 2001; Hallgren et al., 2002; Pear and Crone-Todd, 2002; Vogel and Wood, 2002; Yazona et al., 2002), but one question still remains, Will these tendencies diminish the gap between the developed and underdeveloped areas or will they increase it?

The purchase and use of high-end equipment in scientific research is not uncommon, even in some regions of underdeveloped countries. However, having access to the high-quality images produced by such sophisticated equipment is not an easy task. Usually, students have infrequent contact with a light microscope and very limited experience with electron microscope images, often confined to those found in textbooks. This situation is even worse in less-developed regions. Owing to resource limitations, both financial and material, some university courses cannot afford to purchase either microscopes or video-microscopes to be used in the practical histological and pathological classes.

To present information in a more democratic way, allowing the biomedical student or even the average citizen to have access to high-quality "scientific-type" image, a free histological database was developed. To accomplish that, digital controlled microscopes were used in association with digital image processing software and Intranet or Internet. In a first step, the production of digital image montages (as shown in Figure 1), allowed the visualization of high-resolution wide-field frames (Figure 2). The advantage of such montages is that they help in the observation of detailed structures (normally observed only at higher magnification) and associated details, cells or tissues that are normally visible in low-magnification images (Wootton et al., 1995; Monteiro-Leal et al., 2003). The students easily associate the montage viewed on the computer monitor, or on the television, to the morphology described by the professor in the theoretical lectures or previously read in books, but now, as mentioned before (Vichitvejpaisal et al., 2001), with a far superior quality, when compared with the normal textbook images.

The production of a free Web-based Digital Histology Atlas (Figure 3), organized with images created by the use of state-of-the-art equipment and computer montages, is allowing students from different regions to acquire greater amounts of visual information. The students have accessed the Atlas using personal computers in the university, in their homes or in public places, such as Internet cafes.

The production of a free Web-based Digital Histology Atlas is allowing students from different regions to acquire greater amounts of visual information.

On the other hand, as confirmed by the graphics in Figure 4, our work suggests that the Web-based distance education is not bringing about a reduction in the learning discrepancies between the developed and the underdeveloped regions. This finding is reflected by the incomparably greater number of visitors to our Web site who come more from developed regions, both in Brazil (regions South and Southeast) and the rest of the world (the access from Europe plus USA and Canada totaled 64% of the foreigner visitors to the Atlas). The limited number of computers and Internet servers in less-developed regions could explain this finding. Concerning biomedical education, this situation, instead of diminishing it, could amplify the gap, because the professional's ability would be influenced by the quality of the universities' equipment and Internet facilities from which he/she graduated.

One possible solution to the above problem is the association of a computer with Internet connections to the same television monitor used for the presentation of the images observed by video-microscopy. This set-up was used in the practical biomedical classes and was shown to be less expensive than buying several computers and connecting them to the Internet. Moreover, we have found that, when using a computer with video-output and a 29- to 36-inch television, there is no need for expensive data projectors and image quality is not greatly diminished. The implementation of such a system in medical schools located outside the main cities would result in a broader normalization of biomedical education regarding the histology, anatomy, embryology, and pathology classes, thus diminishing the learning gap between these places and the more developed centers.

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