VIRTUAL MICROSCOPY IN HISTOLOGY AND PATHOLOGY EDUCATION
AT THE FACULTY OF MEDICINE, UNIVERSITY OF NOVI SAD

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Summary
Both histology and pathology as a scientific fields and as an educational subjects have always relied on technology. In the 19th century a major breakthrough happened in certain areas related to histotechnology, so histology and pathology were rapidly developing. Technological revolution has lead to modernization of histology and pathology teaching, resulting in virtual microscopy. Advantage of virtual microscopy is an improved way of teaching and better cost-effectiveness. As a method of histology and pathology teaching, it was implemented at the Faculty of Medicine of the University of Novi Sad in 2016, in a specially equipped classroom at the Institute of Histology and Embryology. Virtual Local Area Network segregation is enabled in this classroom, which allows network traffic from different groups of users to be securely segregated, creating an independent environment for each student’s computer. Students can simultaneously view audio-visual contents on their monitors, on projector screen and on 6 large 55-inch screens. Preexisting microscope glass slides with most representative tissue or organ sections and optimal staining quality were selected and scanned with NanoZoomer S210 Digital slide scanner – Hamamatsu that can rapidly scan glass slides and convert them to digital data. For viewing digital slides we use NDP.view2 program. It allows moving, rotating, zooming and focusing of digital slides via the mouse or keyboard. Program enables morphometric measurements, colorful special pointers, annotations on slides and “bird’s-eye-view”.

Key words: Microscopy; Image Processing, Computer-Assisted; Education, Medical, Undergraduate; Students, Medical; Histology; Pathology; History of Medicine; Computer-Assisted Instruction

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Histology before Subject “Histology”

In medical and biological science and education, histology is a relatively new discipline, although it has been studied much earlier than it was named. Centuries ago, from the time of Antonie van Leeuwenhoek (1632–1723), microscopic structure of tissues was recognized and analyzed. Perhaps one of the most important discoveries in this field was a discovery of pulmonary capillary network of frog, by Marcello


Ključne reči: mikroskupija; kompjuterska obrada slike; osnovne studije medicine; histologija; patologija; istorija medicine; edukacija uz primenu računara
Abbreviations
LM – light microscope
VM – virtual microscopy

Malpighi (1628-1694), who was named the “father of histology”. Next man designated as the “father of histology” was Marie F.X. Bichat, who described 21 types of tissues in 1801, but without using microscope. The term “histology” was finally introduced in 1819, by Carl Mayer, professor of anatomy in Bonn (Ueber Histologie und eine neue Eintheilung der Gewebe des menschlichen Körpers) [1, 2].

19th Century Scientific Revolution in Histology

Histology as a scientific field and as an educational subject has always relied on technology. It is believed that first microscope was constructed in 1590, with magnification not greater than 10x, but it was Galileo Galilei in 1609 who explained and implemented the laws of physics in the construction of microscopes [2]. His work was continued through centuries, and the introduction of reliable, high-quality light microscopes more than 150 years ago enabled analysis of tissues and cell structures at an increasingly smaller scale. At the same time, in the 19th century, modernized histological techniques enabled microscopic observations to influence medicine development, practice and education [3]. Histological techniques, particularly paraffin wax sections and staining by haematoxylin (Waldeyer W, 1864), have become developed enough to provide understanding of tissue structure, and pose it as a scientific base of physiology and pathology. All of this has lead to great discoveries in the beginning and middle part of 19th century: cellular theory (Mathias Schleiden and Theodor Schwann), cellular pathology (Rudolf Virchow) and microorganisms as a cause of diseases (Louis Pasteur), which all stand today as foundations of modern medicine [1].

Histology in 19th and 20th Century Medical Education

Histology became part of medical education curriculums at the end of 19th and beginning of 20th century. The first textbook with histology material was published by Jacob Henle in 1841 (Allgemeine Anatomie) and next by Arthur Hassall in 1846 (Microscopic Anatomy of the Human Body in Health and Disease), and the first textbook containing only histological topics and knowledge was published by Rudolf Albert von Koelliker in 1852 (Handbuch der Gewebelehre des Menschen). At this point of time, histological technique was underdeveloped, and their examinations were very simple, on native tissue in unstained slides [1, 2].

Changes in histology course materials at the beginning of 20th century have reflected improvements in histological techniques and slide preparation as well as developments in light microscopes and associated photomicroscopy [4]. At this time, histology textbooks become modern, representing histology as we known today [2]. One of the most famous European textbooks was written by Ladislaus Szimonowitz (Lehrbuch der Histologie und mikroskopischen Anatomie, 1901), from Lemberg (today Lviv, Ukraine), and it was translated to many world languages. One of the world’s most respected textbooks in histology was written by Russian author Alexander Maximow in 1915. It was translated into English language by William Bloom (A textbook of histology, 1930, Philadelphia: W. B. Saunders), and it had 12 editions [1].

Changes in course content during the 20th century initially emphasized new knowledge of structure as observed at the light and electron microscope levels. Along with light microscopy, transmission and scanning electron photomicrographs were used in teaching histology during the second half of the 20th century. Academic teachers subsequently incorporated more histophysiology and histopathology into their courses to emphasize newly acquired information on the function and clinical relevance of the cells and tissues studied [4]. Until today, a series of the most respectable histological textbooks were published in Springer by professor Radivoj Krstić, histologist from Novi Sad and Lausanne, translated into German, English, French, Russian, Italian, Japanese, and Spanish language.

Beginning of Histological Education in Serbia

Histological experimental work and teaching in Serbia started at the Belgrade Higher School (latter developed into University of Belgrade) at the end of 19th century. The first microscopes were transferred from the Palace of King Milan into Belgrade Higher School, so that Živojin Đorđević (1872-1957), the professor of zoology in this school, could start with histological technique and histological education from 1899. Eduard Mihel (1864-1915), the first pathologist in Serbia, started with histological technique in prossecture at the General State Hospital in Belgrade, in 1896. He was interested also in truly histological questions, like neuroglia, modern topic at that time, and published paper about them in Serbian Archive for General Medicine.

Institute of Histology at the Faculty of Medicine in Belgrade, established in 1921 by Aleksandar Kostić, was the foundation for the education of histology in Serbia. Under the influence of professor Pol Bouin from histological schools in Nancy and Strasbourg, professor Kostić introduced modern methods in research and education at the Institute of Histology. From 1921, he published series of histology manuals and textbooks, with many original photographs of histological slides [5]. Finally, all Serbian histologists active today, are disciples of professor Kostić, as they all have studied histology from the last edition of his great textbook of histology in 1980s. One of his apprentices, academician
Virtual and Light Microscopy in 21st Century Medical Education - Advantages and Disadvantages

Long before the availability of colour-printed textbooks and the advent of personal computers and portable electronic devices, the best method by which students learned about histological, biological and pathological entities was by viewing specimens through light microscopes (LM) [6]. Over the last decade new technological advances have resulted in significant changes how we teach histology and pathology, and one of them is the abandonment of LM in favor of digital histological images, referred to as "virtual microscopy" (VM). Students can access these images through local networks or the Internet [3]. More and more institutions of higher education offer histology and pathology courses that partially or entirely rely on virtual microscopy, as a main teaching tool. In 2009, about 50% of pathology courses in the United States already have or expect to implement virtual microscopy [7].

So why is the use of LM no longer "cool" for teaching histology and pathology? Some of the usually mentioned disadvantages of LM are [3, 6]:
- constant financial drain due to maintenance of a large number of student microscopes and sizeable collections of glass slides;
- great disparities between the learning resources that are available to individual students due to variable quality of histological preparations and difficult acquisition and replacement of many tissues, especially of human origin;
- students lack experience in using LM before undergraduate medical school, so their LM usage skills and etiquette are poor, and they need time to master it, but the time dedicated to basic medical-science practical sessions in integrated training systems is insufficient;
- low accessibility to microscopes and slides, due to their limited number or spatial overlapping with other courses (for example, when same classrooms are used by several educational subjects).

It is not only technical and financial aspect that should be taken into consideration, but also a fact that we are teaching a traditional subjects to today's "computer-savvy" generation of students who explore and utilize all possible virtual and electronic resources.

Positive aspects of traditional LM in teaching process are developing skills required to manipulating a LM and to appreciate the variability of the biological material they have at their disposal.

Advantages of VM are numerous, based on pedagogy, efficacy and cost [3, 6, 7, 8–11]:
- provides students with a viewing experience that is very comparable to real histological glass slides;
- every student has equivalent access to the highest quality slide material;
- adequate software allows each student to select specific regions of interest on the slide, to zoom in and out and to move to other areas at their free choice;
- at high magnifications it is easy for the student to maintain orientation with respect to the entire section;
- image is always in focus, with optimized contrast and adjustable virtual illumination;
- use of labels enhances learning process, and many other.

- students and professors adapt very quickly to the use of the virtual microscope;
- single-use microscope laboratory can be converted into a multi-use computer laboratory;
- after an initial investment in the scanning stage, software and servers, the financial and administrative advantages allow enormous economic savings;
- in the long-term with regard to equipment, technical staff and laboratory facilities.

Virtual microscopy has its own drawbacks: it delivers only a single plane of focus, thus lacking the three-dimensionality, which students can obtain using the fine focus knob of a regular microscope. In addition, VM relies heavily on a stable technological infrastructure that must accommodate multiple users accessing the same slide simultaneously and potential server outages [3, 6]. There were also some drawbacks recognized during the hours of histology practical, like a tendency of some students to passively follow the demonstration of digital slides. This should be minimized by the proper interactive engagement of students in the practical histology session [12].

The classic LM enthusiast argues against the use of simulators as VM, claiming that they can fundamentally alter the essence of medical education, and...
in contrast, technology aficionados may be infatuated with new inventions and be too quick to adopt new technologies without validating them. This can cripple students’ abilities to adapt and deal with real-life situations [3]. We believe that adequate monitoring of students’ impressions and performance can prevent such an unfavorable prognosis.

Students’ Impressions and Achievements

As new technology in teaching process is being implemented, students’ perception must be taken into consideration. Study conducted by Holaday et al. at the University of Michigan Medical School (class of 2014), looked at the usage of various electronic and traditional histology-learning resources, and revealed two important tendencies. In general, most students preferred to study histology at their own time and scheduled resources, such as lectures and lab sessions, suffered a decreasing attendance as the academic year progressed. This finding is in accordance of our own observations at the Department of Histology and Embryology. The second major finding showed a strong and growing preference to use a variety of electronic resources, not only VM [13]. In the study of Foad et al. students appreciated the ease of using VM vs. LM and found the former more interactive and that continuous feedback from tutors minimizes boredom and knowledge gaps. Students gained skills for the use of the VM materials swiftly. As the result, the duration of the sessions can be reduced, or students can spend extra time in validating the skills attained. In contrast, the LM group’s skills had a steep learning curve, and often valuable time during the sessions was dedicated to adjusting the microscopes’ fields, power and focus [6]. Our own observations during VM teaching and students’ comments correlate to the results of this study.

Students analyzed by Ostrin et al. declared to have much higher motivation when using VM in histology classes [9]. Questioners used by Anyanwu et al. showed that 78% of students have some kind of problems using LM, 68% of students think they understand histology better when using VM, while 73% believe that they have better chances in passing the histology practical exam if conducted by VM [10].

With the introduction of new technologies such as virtual microscopy the question arises whether students’ performance suffers? Foad et al. compared success of both methods by written and practical exams. Students in the VM group performed better than those in the LM group in both practical and written exams, as reflected by their more-uniform performance and less-scattered grades. The virtual microscopy group had the advantage of optional online off-campus access to study materials, which they spent an average of 2.5 h reviewing [6]. Study by Anyanwu et al. showed same results in students’ impressions and performance, with the information that the costs of conducting examination using VM were significantly reduced [10].

Collier et al. surveyed teaching assistants for their acceptance of VM use as a teaching tool for undergraduate students in histology. They advocated the use of VM besides providing the students with access to LM. Some researchers affirmed that VM “can effectively replace the traditional methods of learning pathology” [14].

Integration of Virtual Microscopy in Teaching Process at the Faculty of Medicine, University of Novi Sad

Up until 2013 at the Medical Faculty in Novi Sad, classical light microscopes were used to perform exercises in the histology subject. The large number of students distributed by groups, the lack of sufficient histological slides, and frequent microscopic failures were the main reasons for changing the form of teaching. During the next two school years (2013/2014 and 2014/2015), all student microscopes were replaced by a central microscope that, using a microscope camera connected to the computer, enabled the “live” image to be projected onto the projection screen. A similar form of teaching is present today at the Medical Faculty in Nancy, France. This type of teaching enabled the trainer to achieve greater interaction with students. However, this mode of teaching also had some technical shortcomings. One of the greatest shortcomings is that students that are the farthest from the projection screen could not see clearly histological details on the screen due to poor resolution.

During 2015, thanks to Ivan Milenković MD, PhD, an assistant professor of the Institute of Neurology Medical University of Vienna, we enabled to scan the entire microscopic collection in high resolution for both Departments, Histology and Embryology and Pathology, respectively. That same year, thanks to the funds of the Provincial Secretariat for Higher Education and Scientific Research, intended for raising the quality of teaching, 25 computers were purchased for analysis of scanned slides. This type of teaching was used during the next two years (2015/2016 and 2016/2017). Faculty of Medicine University of Novi Sad, led by dean professor Snežana Brkić MD, PhD recognized the importance of modernization in histology and pathology teaching methods, and as part of the reconstruction of the Institute of Histology and Embryology, in June 2017 the first modern classroom for performing VM teaching was formed.

Making a classroom adequate and fully equipped for implementation of virtual microscopy required many infrastructural investments: the purchase of specially made tables with 2 places for students with electricity and internet connections; 38 computers and monitors; 6 large screens as well as video projector. VLAN (Virtual Local Area Network) segregation is enabled in this classroom, which allows network traffic from different groups of users to be securely segregated, creating an independent envi-
environment for each group or customer (student’s monitor/computer). Users of this multifunctional classroom can simultaneously view audio-visual contents on their monitors, on projector screen and on 6 large 55-inch screens (Figure 2).

Such equipped classroom is optimal space for theoretical or practical lessons and practical exams in histology and pathology. Moreover, due to its multifunctionality, it is suitable for all kinds of presentation. In honor of the reestablishment of the Institute of Histology and Embryology at the Faculty of Medicine University of Novi Sad, a symposium “News in Histology and Embryology” was held in June 2017, and it went perfectly. This has established another possible usage of such multifunctional classroom.

For making virtual microscopy slides we used preexisting microscope glass slides of different tissues and organs. These slides were used as teaching collection for students, while light microscopy was used as a method of teaching. Slides with the most representative tissue or organ sections and optimal staining quality were selected and scanned with NanoZoomer S210 Digital slide scanner - Hamamatsu which is whole slide scanner that can rapidly scan glass slides and convert them to digital data. This scanner can automatically scan up to 210 slides. Dimensions of compatible glass slide are 26 x 76 mm with thickness from 0.9 to 1.2 mm. Scanning resolution on this scanner for 20x mode is 0.46 μm/pixel (with scanning speed of approximately 60s) and 0.23 μm/pixel for 40x mode (with scanning speed of approximately 150s) [15].

For viewing digital slides we use NDPview2 program. This software is used for displaying digital slide files of histopathological samples (NDPf, NDPI files, VMS files and VU files) that have been created on the Hamamatsu NanoZoomer system. It allows more responsive and smooth panning (moving and rotating), zooming and focusing functionality via the mouse, keyboard or other devices of various digital slides. Within the program, it is also possible to perform morphometric measurements, set of colorful special pointers and annotations on slides, as well as the ability to store images.

Figure 2. Nowadays virtual microscopy classroom

Slika 2. Današnji izgled virtuelne mikroskopske učionice

Figure 3. Screenshots of NDP view 2 software in different magnifications with labels and annotations

Slika 3. Prikaz NDP-view 2 softvera pri različitim uveličanjima sa oznakama i prilepšanjima
in different formats (.jpg, .bmp, .tiff). Also NDP.view2 offers a “birds-eye-view” specific only for this software (Figure 3) [16]. Today, a collection of scanned digital slides of the Department of Histology and Embryology and the Department of Pathology used in practical exercises counts 200 slides.

Our Future Goals

After successful technical implementation of VM in the Histology and Embryology and Pathology courses, other goals must be achieved. As stated above, careful monitoring of students’ impressions on both good and bad sides of VM must be observed. Analysis of exam performance, questionnaires on students’ satisfaction and assessment of knowledge retention are also planned.

Since our institution provides integrated studies in Medicine and Dentistry organized in English language, many students are not citizens of Serbia so e-learning and broad on-line availability of virtual slides are of exceptional significance. And finally, since the pace of change in education and technology is not slowing down, development of novel interactive teaching tools and approaches that are highly valued by today’s students will be our point of interest.

Conclusion

Despite potential shortcomings, when compared to the traditional, microscope based approach of teaching histology and pathology, virtual microscopy is at least comparable, if not more effective. Most students and academic teachers appear to have enthusiastically embraced this change of teaching modus with no indication that learning success has been compromised. When carefully used in the context of a coherent didactic program the advantages of adopting virtual microscopy and other electronic educational media clearly outweigh their limitations.

However, the knowledge of how to operate a regular light microscope is still a useful skill. This not only applies to the research environment, but also to some clinical settings, especially pathology, microbiology, embryology and others.

References


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