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Improving the Teaching of Histology by Using the Manual Whole Slide Imaging Technology

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Abstract—The tissue sample analysis based on the visualization of the entire tissue sample represents an important methodology in clinical diagnostics, research, and education. This process that enables visualization of the entire tissue sample is named whole slide imaging. For clinical purposes, professional and very expensive scanners are used for tissue sample digitization and the creation of whole slide images. In education, it is possible to use whole slide images that do not necessarily follow strict medical standards and regulations. Therefore, the important research task is to propose a methodology for the manual creation of whole slide images and its adequate use in medical education. In this paper, the use of manually created whole slide images in histology classes education was analyzed. In the experimental part of this research, the methodology of manual creation and usage of whole slide images (WSI) in histology education is presented in more detail. The subjective assessment of the user's satisfaction with WSI usage in education was also performed. It was shown that the whole slide images were evaluated very positively in the histological analysis and education process. Using WSI can significantly improve histology teaching, especially when distance education principles are implemented. Therefore, in crises like the pandemic situation COVID-19 WSI usage becomes a very important educational tool for teaching microscope-related topics. The results of this research showed that the use of whole slide images in histology classes is in line with the process of digital transformation in education.

Keywords- whole slide imaging, virtual microscopy, histology, user experience, distance learning

I. INTRODUCTION

Conventional light microscopy represents the fundamental principle for analyzing tissue samples in histology, pathology, and other related disciplines. In recent times, the new methodology for the analysis of the samples is based on principles that enable the display and visualization of the entire tissue sample. Whole slide imaging is based on the creation of one high-resolution image that visualizes the whole slide. The result of this process is a digital image called the whole slide image.

Recently, the term whole slide imaging is increasingly replacing the term virtual microscopy. The essence of the principle is that the entire preparation is scanned and displayed on the computer monitor with the use of appropriate software. The digital image that is obtained in this way contains a large amount of information about the morphological and functional characteristics of the tissue sample. Therefore, the WSI provides the basis for further efficient analysis of the scanned tissue samples. Professional and very expensive scanners are

mainly used for tissue sample digitization and the creation of WSI. The usage of those scanners provides WSI with extremely high resolution and information quality. The WSI obtained in this way can be used for various purposes such as initial diagnostics, exchange of digitized preparations, quality analysis of samples, digital archiving of preparations, scientific research, and education.

Because professional scanners for creating WSI are very expensive, one of the recent research tasks is to define a methodology for manually creating a high-resolution WSI. This task is based on the use of many images, whose quality would correspond to a specific purpose, and that can be stitched into one high-resolution digital image. This image represents a manually created whole slide image. If the WSI is used for the medical diagnosis or archiving of tissue samples, the use of professional scanners is necessary due to the standardization, legislative, quality, and efficiency of analysis. But in the field of education, where previously listed requirements are not mandatory, the use of manually created WSI is possible and valid.

The previous research showed that WSI can be efficiently used in virtual classrooms and digital collaboration between teaching staff and students at different levels of education [1, 2]. Significant technological achievements have led to changes within the educational process and the usage of different software applications to improve teaching. Digitization has become a necessary part of the teaching process, especially during the COVID-19 pandemic. Certain areas, including medical education, were forced to make drastic changes in everyday work to overcome the pandemic crisis [3, 4].

Thus, in special circumstances, the digital teaching process has become the only way to maintain the continuity of teaching. E-teaching, as a relatively new concept, encounters different reactions within the teaching process, as evidenced by numerous types of research on this topic [5]. The implementation and analysis of interactive software in the online teaching of histology was the subject of a study in previous research [6]. In this paper, one of the research objectives is the analysis of the possibility to use manually created WSI in the teaching process of histology.

II. PRINCIPLES OF WHOLE SLIDE IMAGE CREATION

Considering the various areas of WSI application, especially for diagnostics and medical consultations, it is necessary to highlight the importance of standardization and requirements defined by specific legislation. In the context of clinical application, especially diagnostics in the field of pathology, all stages, from the creation to the use of WSI, must be in line with the appropriate standards and legislation. This implies that all accompanying hardware and software equipment must fulfill prescribed standards and regulations. In previous research, different approaches for creating WSI were analyzed [7]. For the process of histology education, some of the legislation and standards issues are not mandatory, so the process of creation of WSI can be implemented with less rigorous conditions and using the existing laboratory equipment.

A. Whole slide image scanners

Whole slide image scanners are robotic microscopes that can automatically scan tissue on glass slides and create a high-resolution composite image using appropriate software [8]. The technical issues of the first WSI scanner types were that the scanning process took too long and that some parts of the samples could not be scanned and recorded. The development of technology has overcome this problem and today's scanners perform the scanning process within a few minutes with a high scanning resolution. There are various models of WSI scanners, and they can be classified among themselves depending on different characteristics such as optical resolution, 2D or 3D recording capabilities, number, and size of glass slides for scanning, image quality, scanning speed, ease of use, reliability, and support for a certain format [9, 10].

The basic components of a WSI scanner are a robotic section that is used for the manipulation of slides, a microscope with appropriate objectives, one or more digital cameras, a suitable light source, and a professional computer. The computer has installed software for managing, manipulating, and viewing digital images. After the initial quality control of the tissue samples on glass, the scanning process begins with

manipulating samples on glass by a robotic arm and placing them in the appropriate positions for scanning.

When the sample is placed in the appropriate location, the scanning process is performed according to the predefined set of parameters. The scanning process depends on the principle of using the robotic system and the method that will be used to combine individual images into one unique image. The objective characteristics are the key elements of the digital microscope in the optical system. The characteristics and performance of the lenses strongly affect the quality and resolution of the final WSI image. Depending on the region of interest of the tissue section on the glass slides, different magnifications can be used: x40, x50, x100, x200, and the like. The computer and appropriate software are necessary for further viewing of images, especially the usage of numerous services from observation at different magnifications to image analysis.

Fig. 1 illustrates the process that consists of the creation of WSI, their archiving, and usage by remote administrators or users.

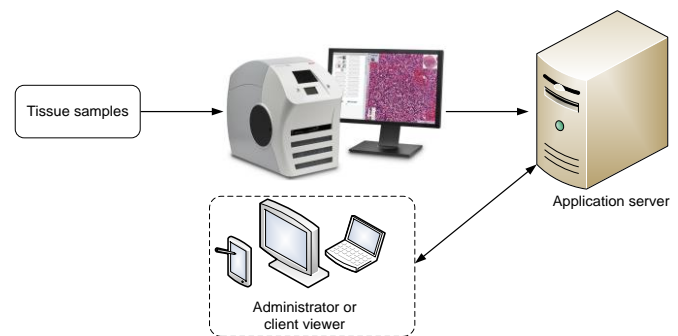


Figure 1. Illustration of whole slide image scanner usage

Regardless of the high image quality, the use of WSI for diagnostic purposes is inevitably accompanied by a certain percentage of error compared to the analysis of the original tissue sample on the glass using a microscope. One of the errors is caused by the fact that some small areas of the tissue sample cannot be scanned. Therefore, it is necessary to identify important areas of interest in the tissue sample and the minimum number of areas of the tissue sample that must be scanned. An important aspect of WSI usage is subjective user experience. Some physicians often prefer direct analysis of the region of interest using classical microscopy of a tissue section on the glass slides rather than the use of digital WSI.

B. The software applications for WSI creation

Considering the high price of professional WSI scanners, one of the research and professional objectives is to propose alternative solutions for WSI creation. The aim is to propose an appropriate methodology for creating a WSI by stitching many digital images that are representing the regions of interest in the observed tissue section on the glass slides. This research task is in line with the trend related to the development and improvement of image processing and analysis software.

There are various software solutions available to apply concepts of image manipulation, stitching, segmentation, and processing, but they differ in the different degrees of user involvement in the process of analysis and processing. Some of the software has built-in algorithms for processing, where the

user performs a minimum number of operations. This type of software can be used for the identification and classification of stained areas and objects of interest on the tissue sample, measuring the density and size of objects of interest, etc. Because the involvement of the user in the process of analysis and processing is relatively small, this software has a higher error level, and they are suitable only for automated and routine analyzes.

For more complex and precise research and professional studies, it is necessary to use programs that require greater interaction with the user. Intensive interaction with the user, the process of analysis and processing using software is guided in the desired direction and the error level can be significantly reduced.

There are software applications that can be used to create WSI by applying the predefined methodology and existing laboratory hardware equipment. By using an appropriate microscope and digital camera, the microscopy process can be adapted to the requirements of the software, and as a result of the final software processing, a WSI with satisfactory quality can be obtained. The previously described procedure is often called manual whole slide imaging. Therefore, instead of the automated scanning process used by a professional scanner, a microscope, and appropriate software can be used to obtain WSI.

A good example of this methodology is the use of Microvisioneer software for manual whole slide imaging. The use of Microvisioneer software is based on its connection to a standard optical microscope with the addition of a suitable camera [11, 12]. The first step consists of an initial adjustment of the microscope parameters. After that, the digital image recording is performed by moving the tissue section on the glass slides. Within this step, the continuous acquisition of digital images is realized. After appropriate software processing of an obtained set of digital images, the result is the WSI of the tissue sample. It is obvious that the adjustment of optical parameters is the first important prerequisite for the acquisition of digital images. Their appropriate stitching and the elimination of overlapping regions of interest are performed by the software application [13].

The obtained WSI can further be used for various analyses, with the possibility of sharing and collaboration between users which is especially useful for distance education in the area of medicine.

C. The main characteristics of the whole slide images

After using a professional scanner or one of the dedicated licensed software solutions, WSI files with adequate quality are obtained. If the resulting WSI files are in line with all the necessary standards, their size is usually in the order of megabytes or several gigabytes. The main characteristics of WSI are image dimensions, file size, type of compression, color model, etc. With the increasing sample size and image resolution, the size of the final file is significantly increased. For this reason, it is necessary to use compression for viewing and manipulating files. Depending on the area of application, lossy (JPEG, JPEG200 format) or lossless (TIFF format) image compression can be used. In some situations, especially when the main purpose of digitization is archiving, it is preferable to use lossy compression. The main issue is to preserve the

quality of observed diagnostic information of interest. That information must not lose its quality [14].

It is important to note that the different coloring of the region of interest in the tissue sample has a strong influence on the sample assessment and user experience, which affects the diagnosis. For this reason, the preparation of samples for scanning is subject to standardization and regulation. Besides that, the samples are viewed on the monitor screen, and it is necessary to carry out the appropriate calibration of the scanner and the monitor screen using reference samples. In this way, a consistent display of the used color is obtained independent of the scanner and the monitor type [10].

Because WSI files require larger memory capacities, the problem of efficient loading during viewing and analysis is often present in the case of remote users. There are several ways to overcome this problem but all of them are usually based on the principle of the pyramid of pictures obtained with different magnifications [14, 15].

For faster and more efficient work, each WSI can be divided into smaller segments, square or rectangular in shape. Each segment is associated with an area within the original image. Their loading ensures faster and more efficient work and avoids loading a large amount of data. Another approach to WSI display optimization is based on saving the image in multiple files of different resolutions, which is often called an image pyramid. By choosing only one image that contains only the desired information, the need to upload large files is reduced.

Fig. 2 illustrates the principle of the pyramid of pictures. Digital slides are stored using the concept of the pyramid of pictures obtained using different magnifications. It provides the possibility to load appropriate pictures with magnification that enables visibility of the needed histological structure.

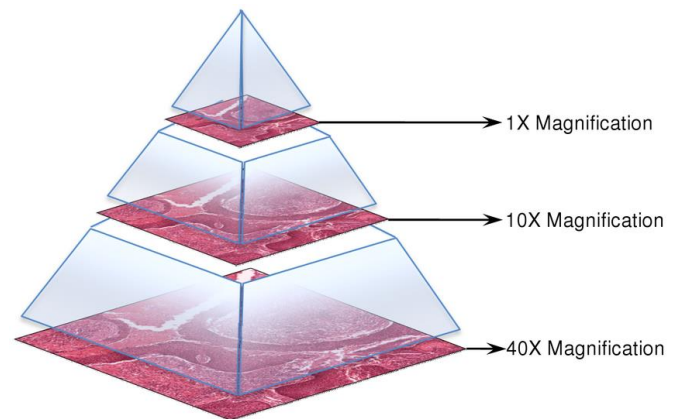


Figure 2. A pyramid of images that consists of images with different resolutions [15]

Digital slides are stored using the concept of the pyramid of pictures obtained using different magnifications. It allows the loading of appropriate pictures obtained with necessary magnification, enabling visibility of the wanted histological structure. Fig. 2 presents the concept of the pyramid of pictures obtained with the magnifications 40x, 10x, and 1x. Using the magnification of 40x a single digital image is obtained, while usage of magnifications of 10x and 1x many digital images will be obtained to represent some area within the histological slide.

This methodology is used to obtain multi-layered images with high resolution that can be stored in a pyramidal manner. This pyramidal file represents the whole slide image and should be opened and visualized using the appropriate software. The development of different possibilities of software solutions for visualizing and processing the WSI represents an important research task in whole slide imaging.

III. THE WHOLE SLIDE IMAGING FOR HISTOLOGY EDUCATION

Professional WSI scanners are very expensive, and their full application is in the field of medical diagnostics. In the educational process, the WSI scanner represents a serious and often unnecessary high investment. In the case of medical education, alternative solutions for creating WSI should be proposed. The methodology of the use and analysis of histological samples in the educational process is not defined by strict standards and regulations, as it is in the field of clinical medical diagnostics. Therefore, one of the modern research tasks is a methodology of manual creation of WSI and its application in the field of medical education. Manual whole slide imaging represents a cost-effective solution to enable the digitization of histology education.

Although WSI scanners provide high-quality images, there are some disadvantages related to technical and especially financial aspects for use in educational purposes. The professional WSI scanner is based on the trinocular microscope with robotic controls so is usually very expensive. Sometimes, as it is in education, it is useful to have more control in creating the high-resolution image. The manual process of creating the WSI can be based on scanning the multiple disjointed areas of the sample and saving the images in different file formats. The concept of manual WSI allows upgrading existing microscopes to provide good enough quality WSI at affordable prices.

Virtual microscopy implies that the WSI refers to the use of a complete microscope slide for creating a single high-resolution digital file. Some areas of interest in the microscopic slide can not be viewed within the one microscopic field under chosen magnification, also. That area of interest within the histological sample must be represented by multiple digital images that will be stitched into a single high-resolution image. Therefore, for representing the larger area of interest with one high-resolution image, the concepts, and methodology of whole slide imaging will be used.

The practical aspects of whole slide imaging are based on the use of appropriate software that enables efficient zooming, finding, and identification of objects of interest in the observed histological sample. In previous research, different software tools were used to enable the simulation of the principles of classical microscopy, which is based on the use of an optical microscope. With the appropriate software solution, it is necessary to simulate the navigation and search of the histological sample, and the simulation of optical magnification to identify the object of interest [16, 17].

In the practical part of this research, Leica's software Aperio ImageScope 12.4.6 was used [18]. This software enables the previously mentioned functionalities necessary for practical research. In addition to the features necessary for the implementation of the experimental part of this research, the Aperio software has several other very useful features. The

adjustments for contrast, brightness, and gamma, viewing and managing of annotations, and extraction of selected regions of a WSI to different file formats are some of them.

With the use of the mentioned software, the respondents performed the whole slide imaging tasks, and therefore it was used in the practical examination of subjective satisfaction with the use of the whole slide image. An illustration of the possibility of navigating to a part of the WSI, adjusting the desired magnification, and identifying objects of interest using the Aperio ImageScope software is shown in Fig 3. The ImageScope main window pane is positioned in the center. In the upper left corner, the zoom slider is illustrated as well as the thumbnail in the right upper corner. The thumbnail window is used as a navigation tool for selecting the desired window within the WSI.

The magnifier is presented in the lower-right corner of the figure. Using the magnifier window it is possible to magnify some object of interest within the WSI.

In the experimental assessment of subjective user satisfaction with WSI usage, 20 students who attend a course in histology participated. They used the previously described Aperio software to simulate the process of microscopy of histological preparations.

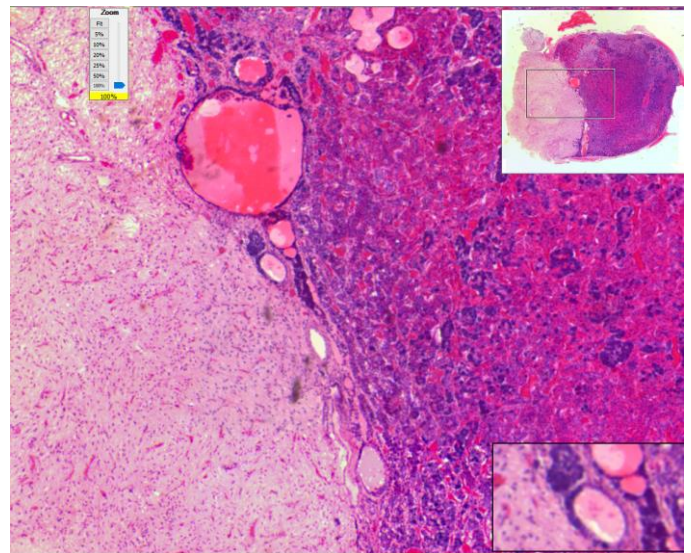


Figure 3. Illustration of navigation simulation, zoom adjustment, and identification of objects of interest using Aperio ImageScope software.

IV. THE MANUAL CREATION OF WHOLE SLIDE IMAGES FOR HISTOLOGY EDUCATION

In this paper, the research focus is the usage of manually created WSI on histology classes. The histology tissue samples have different types of information that should be precisely identified and analyzed, so WSI usage can improve the efficiency of the learning process.

The focus of this research is WSI usage for efficient identification and analysis of histological substructures for education purposes, not WSI usage for medical diagnostics. Therefore, the quality of manually created WSI is good enough for the realization of the education process in Histology classes.

The WSI used in the experimental part of this research was created at the Department of Histology and Embryology, Faculty of Medicine, University of Banja Luka. The laboratory of histology is fully equipped with all the necessary equipment that was used for microscopy and digitization of preparations. In the experimental part of this research, the Leica DM6000 B microscope with a Leica DFC310 FX digital camera was used for tissue sample microscopy. For digitization and archiving the personal computer was used with image analysis software Leica LAS V4.3.

The methodology of manual WSI creation is based on two key phases. The first phase consists of two tasks. The first task is the process of microscopy of the tissue sections on the glass slides where the regions of interest are identified, and the second one represents an initial digitization of previously identified regions.

In this phase, it is necessary to define a pattern that will be used and followed during the capturing process of regions of interest. The patterns that were used in previous research were based on the linear movement of the tissue sections on the glass slides, and one of the often-used patterns is illustrated in Fig. 4 [19]. The same pattern was used in this research.

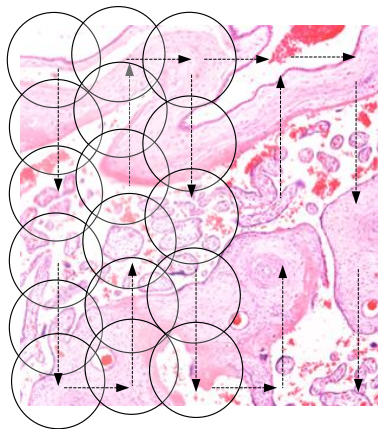


Figure 4. Illustration of pattern used for capturing and digitization regions of interest in the histological sample

The regions of interest should overlap each other to digitize the entire area of the sample. The process of successive microscopy with selected magnification and capturing of regions of interest that overlap each other enables the digitization of all useful information that is contained in the histological sample. As a result of this phase, many images that represent the region of interest captured using a predefined pattern are obtained.

In the second phase, the images that represent regions of interest in the histological sample are stitched into one unique image. The resulting image represents the WSI. The stitching process can be realized using much different software but, in this research, Photoshop CS 2018 is used. A similar methodology was used in the previous research related to manual whole slide image creation [19].

The microscopic images of the region of interest in the histological sample under specific magnification were digitized and saved in a tiff file format. The contrast and white balance of the images were good enough, so there was no need for additional processing to perform the next steps for the manual

creation of the WSI. In this work, the stitching of individual images was realized using the Merge command with additional necessary command options in the Photoshop software. This process results in a whole slide image that represents an entire histological sample or specific area in the sample under chosen magnification.

The process of image stitching is finalized with manual supervision of the edges of the images that were stitched. The supervision of the efficiency and accuracy of image stitching was performed jointly by two persons that are experts in image processing and histology analysis. In the case where the corrections were necessary, they were jointly performed, and the stitching process was again revised and controlled.

The quality of digitization depends on several factors. The quality of the WSI format, used compression, color quality, and poor focus on the objects of interest are some of the important issues. In the case of the use of WSI for education purposes, those factors can be controlled to obtain good enough quality of WSI. Considering the file sizes associated with whole slide images, aspects of use related to efficient loading, storage, and transmission of these images for remote users should be considered also.

In this research, the activities were performed in the laboratory and classroom environment so the tiff file format was successfully used. The previous research showed that the usage of lossy compression, to ensure reasonable file sizes, has no strong impact on the image quality. Therefore, in the case of remote usage of WSI for education purposes, the JPEG standard can be efficiently used [20].

In the experimental part of this research, the placental histological sample was analyzed. For the digitization of the placental histological sample and the different areas of interest, magnifications of x50, x100, and x200 were used. First, the whole slide image was created using this histological sample and x50 magnification. Then, in the placental histological sample were identified areas that were used for the creation of corresponding whole slide images using x100 and x200 magnifications. Therefore, the whole slide images of the chorionic plate under x100 and x200 magnification, the villi and intervillous space under x100 and x200 magnifications, and the basal plate under x100 and x200 magnifications were created. Using the pattern illustrated in Fig. 2, for every identified area in the placental histological sample were obtained digital images that will be used in the stitching process. After the stitching process, the seven WSI were obtained.

The result of the previously described methodology is illustrated in Fig. 5. Fig 5a presents the WSI of the placental histological sample under x50 magnification. Also, in Fig. 5a are marked the areas of interest that were used for the creation of WSI that represent substructures of the placental sample as the chorionic plate, the villi and intervillous space, and the basal plate. The WSI of the chorionic plate under x100 magnification and x200 magnification are illustrated in Fig. 5b and Fig. 5c, respectively. The WSI of the villi and intervillous space under x100 magnification and x200 magnification are illustrated in Fig. 5d and Fig. 5e, respectively. Finally, the WSI of the basal plate under x100 magnification and x200 magnification are illustrated in Fig. 5f and Fig. 5g, respectively. Images obtained in this way were used for whole

slide imaging, to simulate classical light microscope-based histological analysis.

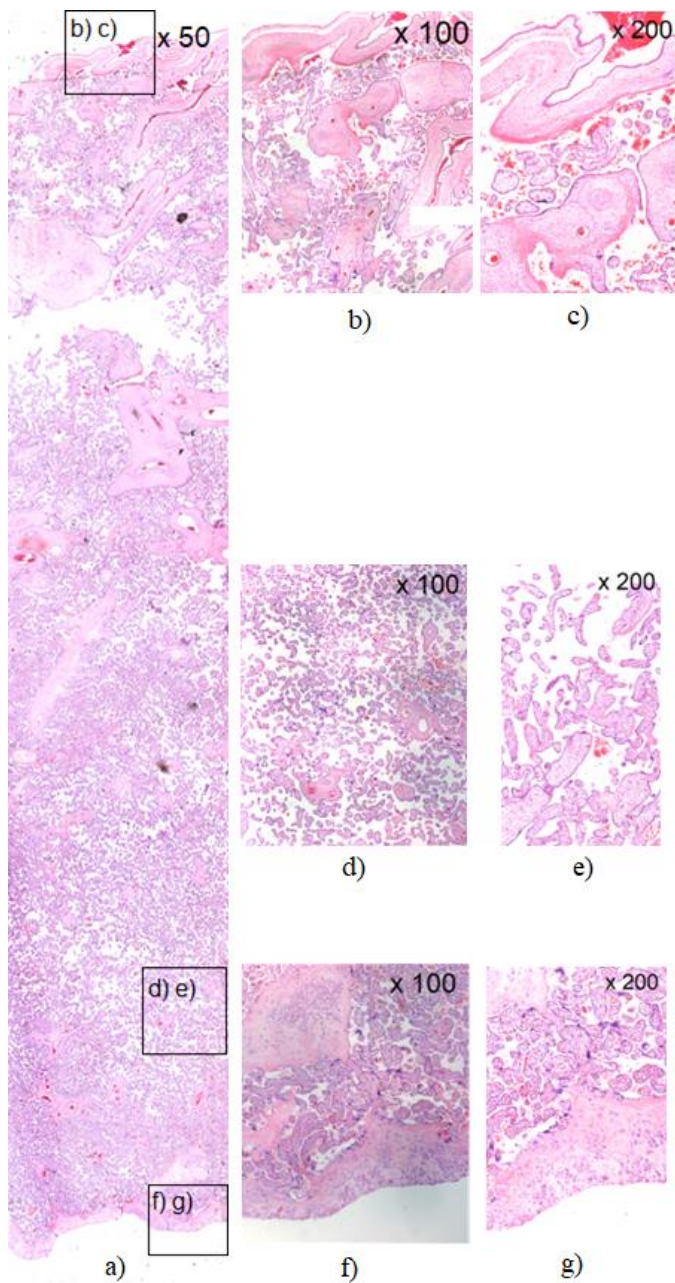


Figure 5. The illustration of the principle for the creation of whole slide images

In the process of manually creating high-resolution whole slide images, a few technical challenges were encountered. The pattern used for capturing and digitizing regions of interest in the histological sample must be carefully defined to enable the digitization of all information within the analyzed sample. The solution is to use a pattern that allows adequate overlapping of the regions of interest. The stitching process of the obtained images that represent regions of interest can be performed using different software solutions. Fine-tuning of the edges of the stitched images should be performed also. In this research, the analysis of available software solutions was not the focus. For both previously described tasks, the Photoshop software

was used with excellent results. For practical use of the WSI the file format is very important, so in this research, the tiff file format was used. The tiff format provides the flexibility for further use and analysis of WSI, as well as the implementation of WSI using the multi-resolution pyramid of images.

V. THE ANALYSIS OF WSI USAGE IN HISTOLOGY CLASSES

Histological analysis is used for qualitative and quantitative analysis of tissue for research, education, or diagnostic purposes. In order to identify histological structures different staining methods can be used. For example, using appropriate staining the cytoplasm of cells and the nucleus will be shown in different colors. Therefore, different colors of histological structures are used for identification and analysis.

Histology is one of the basic disciplines of courses in medical education. The development of technology is the cause and improvement of methods and methodology in the teaching of histology. Besides the methods of optical microscopy in teaching histology are often used techniques of virtual microscopy known as whole slide imaging [21].

A. Histological analysis of the placenta sample

Histological analysis is used for qualitative and quantitative analysis of tissue for research, education, or diagnostic purposes. In order to identify histological structures different staining method is used. For example, using appropriate staining the cytoplasm of cells and the nucleus will be shown in different colors. Therefore, different colors of histological structures are used for identification and analysis. Histology is one of the basic disciplines of courses in medical education. The development of technology is the cause and improvement of methods and methodology in the teaching of histology. Besides the methods of optical microscopy in teaching histology are often used techniques of virtual microscopy known as whole slide imaging [21].

The practical use of WSI in the teaching of histology classes is based on the usage of computers and appropriate software for the visualization and analysis of digitized histological tissues. In that way, every computer with appropriate software for the visualization of whole slide images can simulate a conventional light microscope.

In this research histological sample of placenta tissue was used. For virtual microscopy or simulation of optical microscopy techniques Leica's software Aperio ImageScope 12.4.6 was used. The Aperio ImageScope software is used for navigation to a specific part of the WSI, as well as adjusting the magnification and identifying objects of interest within the area of the WSI. An adequate introduction to whole slide imaging and an explanation of tasks that participants should complete within the histological analysis was performed. With simplified and quick explanations teacher is leading the students to identify the position and characteristics of objects of interest within the area of the histological sample.

Histological analysis based on the WSI of the placenta sample was performed in three phases. First, the parts of the placenta sample that can be differentiated under x50 magnification were explained to students and analyzed. Therefore, the WSI that represents the entire placenta sample, which was obtained by stitching the digital images of

microscope fields of view under x50 magnification was analyzed.

In the second phase, the parts of the placenta that can be differentiated under x100 magnification were analyzed. The selected areas of interest within the placenta sample were represented by WSI obtained by stitching the digital images of adequate microscope fields using x100 magnification. The obtained WSI represents the chorionic plate, villi and intervillous space, and the basal plate under x100 magnification.

In the third phase, the substructures of the chorionic plate, villi, intervillous space, and basal plate that can be differentiated under x200 magnification were analyzed. Those substructures were analyzed using the WSI obtained using the previously described procedure which was used in the second phase. An example of the analyzed substructures is amnion epithelium. This substructure can be well differentiated under x200 magnification in the area that represents a chorionic plate within the placenta sample, Fig. 6. For further analysis of the substructures of interest, the digital zooming of the WSI can be performed, and the other objects of interest can be adequately visible.

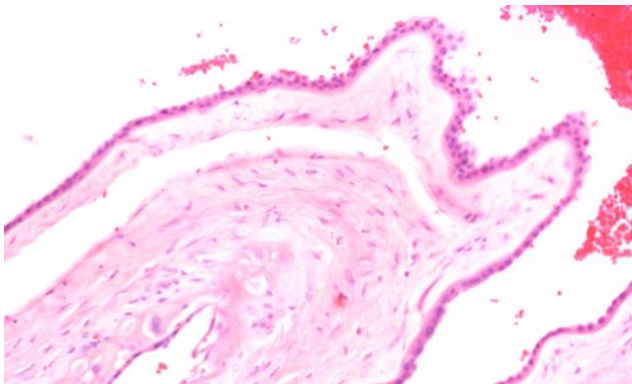


Figure 6. The amnion epithelium under x200 magnification

B. Subjective assessment of the user's satisfaction with WSI usage

The traditional learning method of histological analysis based on optical microscopy has been constantly supplemented with virtual microscopy techniques, i.e. whole slide imaging. Newer researches also show the need for increasing digitization of the teaching process and, therefore, the application of WSI. Some of the research shows that students are very interested in the usage of digitized samples and overall the use of whole slide imaging techniques in learning histology [22].

Therefore, the subjective assessment of the user's satisfaction during the use of WSI was also performed in this research. Participants in the subjective assessment are students that were enrolled in a histology course. All students that participated in the experimental part of this research have the necessary prior knowledge related to the optical microscopy process and the analysis of histological characteristics of a tissue section on the glass slides.

During the practical laboratory exercises in histology classes, the students have been introduced to the principles and basic concepts of whole slide imaging. Students have been

introduced to the Aperio ImageScope software to perform virtual microscopy tasks.

The usage of WSI, zooming in on the region of interest, and identifying objects and characteristics of the histological sample that is the subject of analysis were presented to the students. Students performed the histological analysis that is described in the previous subsection.

A group of 20 students attending the histology course participated in a subjective assessment of the user's satisfaction with WSI usage. Practical testing of user satisfaction consisted of two phases. In the first phase, students used manually created WSIs to identify and analyze objects of interest in the 7 different whole slide images. The creation and characteristics of the WSI that were used in the process of assessment of the user's satisfaction are presented and described in the previous section. Visualization of histological substructures within WSI was performed using Aperio ImageScope software.

The subjective assessment of user satisfaction was focused on satisfaction with WSI usage in the education process, but not on the assessment the of whole slide image quality for medical diagnostics. After using WSI and performing the predefined tasks within histology analysis students took part in the user satisfaction survey. The students participated in answering the survey questionnaire via Google Forms. The questionnaire is designed to identify the quality, usability, and efficiency of the whole slide images for education purposes in histology classes. The questions that participants answered during the user satisfaction survey are presented in Table 1.

TABLE I. EXPERIMENTAL ASSESSMENT QUESTIONS

No.	Questions
1	Was the direction for participating in the experiment sufficient?
2	Did you find the quality of the whole slide image sufficient?
3	Were you able to find the object of interest easily?
4	Was it easy to use WSI to perform the histological analysis?
5	Do you think that the usage of the WSI for educational purposes is efficient?

The Mean Opinion Score (MOS) has become a very popular indicator of user satisfaction in different applications. Therefore, the standard practice for relative subjective scoring of the whole slide imaging is also based on the MOS.

According to Recommendation ITU-T P.910 and assessment and rating of the quality of experience, a five-level mean opinion score scale was also used in this research. The lowest MOS rating is 1, while the highest is 5, Table 2.

TABLE II. FIVE-LEVEL MEAN OPINION SCORE (MOS) SCALE

Mean opinion score (MOS)	Explanation
5	Excellent
4	Good
3	Fair
2	Poor
1	Bad

The graphical illustration of the result of the assessment of subjective user's satisfaction is presented in Fig. 7.

The obtained results show that the students are satisfied with the introduction to subjective assessment that was done before the experimental testing. The requests for histology analysis and methodology of filling out the questionnaire were clear to all participants. The quality of the WSI that was used in the experiment was also positively assessed. Students have positively evaluated the process of using WSI to perform predefined tasks of histological analysis of the samples. The identification of the object of interest in the analyzed samples was positively evaluated as well. The students positively evaluated the possibility of using WSI in the process of teaching the histology classes.

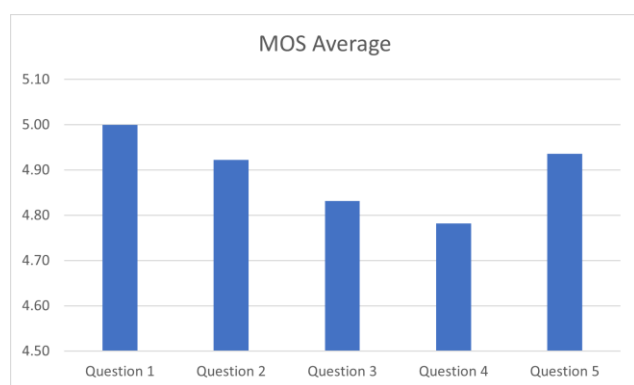


Figure 7. Graphical illustration of the result for assessment of users' satisfaction using the MOS scale

Finally, the analysis of the results of the subjective assessment based on the previously presented questionnaire showed that the whole slide imaging can be a good replacement for conventional light microscopy for educational purposes.

An important research task in the future can be investigating different challenges in terms of improvement of user experience and interactivity when using manual whole-slide imaging for histology education. The influence of different software applications for WSI visualization, using annotation within WSI, the influence of different staining principles to identify objects of interest, etc. can see some of the research goals related to improving the user experience.

VI. CONCLUSION

The visualization of the entire tissue sample provides a comprehensive insight into tissue structure that is often necessary for efficient clinical diagnostics, performing research tasks, or education processes. For accomplishing that kind of visualization of tissue samples, the concept of whole slide imaging is used. The WSI can be obtained using different methodologies. Instead of the use of professional and very expensive scanners for WSI creation, manual WSI creation is often used in medical education.

This research provides a detailed overview of the whole slide imaging and different methodologies for WSI creation. An alternative approach for manual WSI creation instead of the use of professional WSI scanners is presented with more details using real histological tissue samples. The results of the

experimental investigation showed that the manually created WSI has sufficient visual characteristics for histological analysis. Within the subjective assessment, the WSI was evaluated very positively for the histological analysis and education process.

The use of WSI in histology classes is fully compliant with the process of digitization in education. The whole slide imaging can be an efficient replacement option for classical optical microscopy. Considering the previous, distance education in histology classes can be significantly improved using the WSI. This is especially important in a crisis like it was the COVID-19 pandemic. In that situation, the WSI becomes an extraordinarily important tool for education in areas where the one of main topics is microscopy.

Different important issues on whole slide imaging can be interesting for future research. Some of them are the influence of different types of tissue staining, the influence of digital navigation in the identification of objects of interest, the effects of WSI annotation on learning efficiency, etc.

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