15. Pletz J, Sánchez-Bayo F, Tennekes HA. Dose-response analysis indicating time-dependent neurotoxicity caused by organic and inorganic mercury-Implications for toxic effects in the developing brain. Toxicology. 2016;347-349:1-5.
doi: https://doi.org/10.1016/j.tox.2016.02.006

16. Wyatt LH, Luz AL, Cao X, Maurer LL, Blawas AM, Aballay A, et al. Effects of methyl and inorganic mercury exposure on genome homeostasis and mitochondrial function in Caenorhabditis elegans. DNA Repair (Amst). 2017;52:31-48.
doi: https://doi.org/10.1016/j.dnarep.2017.02.005

17. Virgolini MB, Aschner M. Molecular mechanisms of lead neurotoxicity. Adv Neurotoxicol. 2021;5:159-213.
doi: https://doi.org/10.1016/bs.ant.2020.11.002

Стаття надійшла до редакції
16.09.2021

◆

UDC 616-091:57.086.16:004.353.244 https://doi.org/10.26641/2307-0404.2022.1.254314

ANALYSIS OF COLOR PROPERTIES OF RASTER IMAGES OF HISTOLOGICAL MICROSPECIMENS: OWN RESEARCH EXPERIENCE

M.D. Ivanova [1], M.S. Myroshnychenko [2], G.I. Khara [3], O.V. Arseniev [3], V.O. Olkhovsky [2], E.K. Grygorian [2], Yu.Ya. Fedulenkova [2], S.V. Kozlov [4] *

Universta Milano-Bicocca [1]
Milan, Italy
e-mail: mesangium88@gmail.com
Kharkiv National Medical University [2]
Nauky av., 4, Kharkiv, 61022, Ukraine
e-mail: msmyroshnychenko@ukr.net
National University of Pharmacy [3]
Pushkinskaya str., 53, Kharkiv, 61002, Ukraine
e-mail: geoivn11@gmail.com
Dnipro State Medical University [4]
V. Vernadskyi str., 9, Dnipro, 49044, Ukraine
[*]e-mail: tanatholog@i.ua
Університет Мілану-Бікокка [1]
Мілан, Італія
Харківський національний медичний університет [2]
пр. Науки, 4, Харків, 61022, Україна
Національний фармацевтичний університет [3]
вул. Пушкінська, 53, Харків, 61002, Україна
Дніпровський державний медичний університет [4]
вул. В. Вернадського, 9, Дніпро, 49044, Україна

***Цитування:* Медичні перспективи. 2022. Т. 27, № 1. С. 9-15**
***Cited:* Medicni perspektivi. 2022;27(1):9-15**

Key words: *analysis, color properties, raster image, microspecimen, computer program*
Ключові слова: *аналіз, кольорові властивості, растрове зображення, мікропрепарат, комп'ютерна програма*
Ключевые слова: *анализ, цветовые свойства, растровое изображение, микропрепарат, компьютерная программа*

Abstract. Analysis of color properties of raster images of histological microspecimens: own research experience. Ivanova M.D., Myroshnychenko M.S., Khara G.I., Arseniev O.V., Olkhovsky V.O., Grygorian E.K., Fedulenkova Yu.Ya., Kozlov S.V. *This study is aimed to develop a computer program to analyze the color properties of raster images of histological microspecimens used in pathological anatomy and forensic medicine. When developing a computer program, we used the system for building client applications – Windows Presentation Foundation (WPF). The system allows you to create applications with visually attractive user interaction capabilities. The programming language is C#, as well as basic graphics capabilities of the .NET Framework system are used. To speed up the display we applied double buffering. In the course of the research, the authors developed a modern computer program «Analysis of color properties of raster images». This program allows you to analyze the color of each individual pixel of a photograph in RGB and Lab color models, comparing the colors and brightness of individual pixels, selecting groups of points and determining statistical characteristics of them. Characteristic points are well distinguishable in the photographs, studied with the program «Analysis of color properties of raster images». This makes it possible to select and automate these properties, using computer recognition algorithms, completely removing human factor's influence on the analysis results. The computer program «Analysis of color properties of raster images» is of significant scientific and practical interest for specialists both in the field of morphology (pathologists, forensic experts, etc.), and in the field of other biomedical disciplines.*

Реферат. Аналіз кольорових властивостей растрових зображень гістологічних мікропрепаратів: власний дослідницький досвід. Іванова М.Д., Мирошниченко М.С., Хара Г.І., Арсен'єв О.В., Ольховський В.О., Григорян Е.К., Федуленкова Ю.Я., Козлов С.В. *Це дослідження мало на меті розробку комп'ютерної програми, яка б дозволила провести аналіз кольорових властивостей растрових зображень гістологічних мікропрепаратів, які використовуються в патологічній анатомії та судовій медицині. При розробці комп'ютерної програми використовувалася система побудови клієнтських додатків – Windows Presentation Foundation (WPF). Система дозволяє створювати додатки з візуально привабливими можливостями взаємодії з користувачем. Мова програмування – C#. Використано базові графічні можливості системи .NET Framework. Для прискорення відображення застосована подвійна буферизація. Під час проведеного авторами дослідження була розроблена сучасна комп'ютерна програма «Аналіз кольорових властивостей растрових зображень», яка дозволяє аналізувати колір кожного окремого пікселя фотографії в кольорових моделях RGB і Lab, порівнювати кольори і яскравість окремих пікселів, вибирати групи точок і визначати статистичні характеристики вибраних груп. На фотографіях, кольорові властивості яких досліджуються за допомогою програми «Аналіз кольорових властивостей растрових зображень», характерні точки добре помітні. Це дозволяє застосувати для їх виділення алгоритми комп'ютерного розпізнавання й автоматизувати їх вибір, що повністю знімає вплив людського фактора на результати аналізу. Комп'ютерна програма «Аналіз кольорових властивостей растрових зображень» представляє істотний науковий і практичний інтерес для фахівців як в області морфології (патологоанатомів, судово-медичних експертів та ін.), так і у сфері інших медико-біологічних дисциплін.*

Pathological anatomy and forensic medicine are important integral parts of modern theoretical and practical medicine [10, 13]. A single object of research – a corpse brings these branches of medicine together. Microscopic examination of biopsy and autopsy material plays an important role in pathological anatomy and forensic medicine. Its purpose is to identify various general pathological processes and diagnose diseases, determine the time of death and age-related changes, identify a person, etc.

The basic criterion in the foundations of any branch of knowledge is the degree of their quantification, i.e. intensive use of quantitative approaches and mathematical modeling to prove the discovered patterns [4].

Application of various histological, histochemical, immunohistochemical staining methods allows the pathologist and forensic expert to identify the structural elements of cells and tissues, the accumulation of substances in the parenchyma and stroma of organs, characterized by different colors. In this regard, the assessment of the color characteristics of histological microspecimens is of great scientific and practical importance. The visual approach used in science and practice in the study of microspecimens, including the assessment of color characteristics, has a number of disadvantages. They are associated with experience, psychic-emotional state, features of the doctor's visual analyzer. This, however, does not allow us to diagnose accurately and correctly the processes and phenomena in full, to obtain their qualitative characteristics and statistically analyze the data [1, 7]. Morphologists' views can be highly subjective, manifesting not only in visual assessment, but also in the most diverse terminology they use to describe color [3]. Therefore, the search for a promising tool that would allow a correct assessment of the color properties of histological microspecimens is an urgent problem.

The aim of this work is to develop a computer program to analyze the color properties of raster images of histological microspecimens used in pathological anatomy and forensic medicine.

MATERIALS AND METHODS OF RESEARCH

When developing a computer program, we used the system for building client applications – Windows Presentation Foundation (WPF) [14]. The system allows you to create applications with visually attractive user interaction capabilities. The programming language is C#, as well as basic graphics capabilities of the .NET Framework system are used. To speed up the display we applied double buffering. Histological sections of human tissues stained with hematoxylin and eosin were used to test the computer program. Preparation of histological sections was performed in accordance with the standard procedure [9].

RESULTS AND DISCUSSION

Many factors determine the quality of photographs of histological microspecimens (proper implementation of staining techniques for microspecimens; use of standard lighting in a microscope when photographing; resolution of a digital camera, etc.). As we know, resolution in a digital camera rests on the number of horizontal and vertical elements (pixels) of the image that it can capture. The greater the number of pixels horizontally and vertically, the higher the resolution of the camera is and, therefore, the clearer the image and softer color transitions. Modern cameras provide a resolution of 16 million pixels or more. Moreover, the number of colors of each pixel is more than 16 million. A digital image, stored in a computer as individual pixels, is called a raster image.

There are various color models to describe colors, understood as an abstracted depiction of colors in the form of a set of three numbers, called color coordinates. The set of acceptable shades of color and the method for interpreting these data determine the color space [6]. We used RGB, XYZ and Lab color models in our computer program «Analysis of Color Properties of Raster Images» (ACPRI).

Considering that the main modern formats of computer storage of raster images rest on the RGB color model, it is this model that we use in the ACPRI program to obtain information about the color of individual pixels of raster images. The values of the numbers R, G and B are coordinates in three-dimensional Cartesian space and, accordingly, we can calculate the distance between different points in this space [5]. However, this definition of distance can give a small distance for distant points, and long for nearby ones. This drawback makes the calculated distance unsuitable for practical purposes.

The creation of the XYZ color model can be seen as an attempt to eliminate the above mentioned disadvantage of the RGB model [2]. We used the XYZ color model for the calculations associated with the transition from the RGB color model to the Lab color model. Such a calculation is more convenient and allows you to control each step of the program debugging.

The currently recognized as an international standard, the Lab color model, in contrast to RGB, makes it possible to determine accurately differences between the color components of individual pixels of a raster image for practical purposes. These considerations determined the Lab color model in the ACPRI program we developed as the main one.

To calculate the brightness of a pixel in the RGB color model, we used the formula:

$$Y = \frac{0.299 \text{ x } R + 0.587 \text{ x } G + 0.114 \text{ x } B}{255}$$

In the Lab color model, the L component gave the desired value.

When we launch the ACPRI program, a dialog form displays on the computer screen to control the program's operation (Fig. 1). This form includes four components: a field for displaying a photo, a field for a list of selected pixels, information fields and a field for control buttons.

We see a graphic image of the studied photograph of a histological microspecimen in the display field.

Color parameters for each pixel are displayed in the field of the list of selected pixels: (No.) – the ordinal number of the selected pixel; (x) – x-coordinate of the pixel in the image; (y) – y-coordinate of the pixel in the image; (R, G, B) – the values of the color components of the pixel based on the RGB color model. If the information about the next selected pixel does not fit in the designated field, a scroll bar is automatically added to the field (Fig. 2). The ruler allows you to position yourself on any line of the list. The user has the ability to define several groups of pixels by inserting separators into the list (Fig. 2).

Information fields (Fig. 1) display the current state of photo processing. Each information field consists of two elements: the title and the value of the displayed parameter.

Information field «Measure» informs about the scale of the image. After loading the photo, the initial scale is 1. For a detailed examination, it is possible to discretely set the scale (2, 4, 8, 16).

Information fields «xC», «yC» are coordinates of the current pixel.

Information fields «xS», «yS» are coordinates of the sample pixel. The user, being at any point in the image, can define the current pixel as a sample.

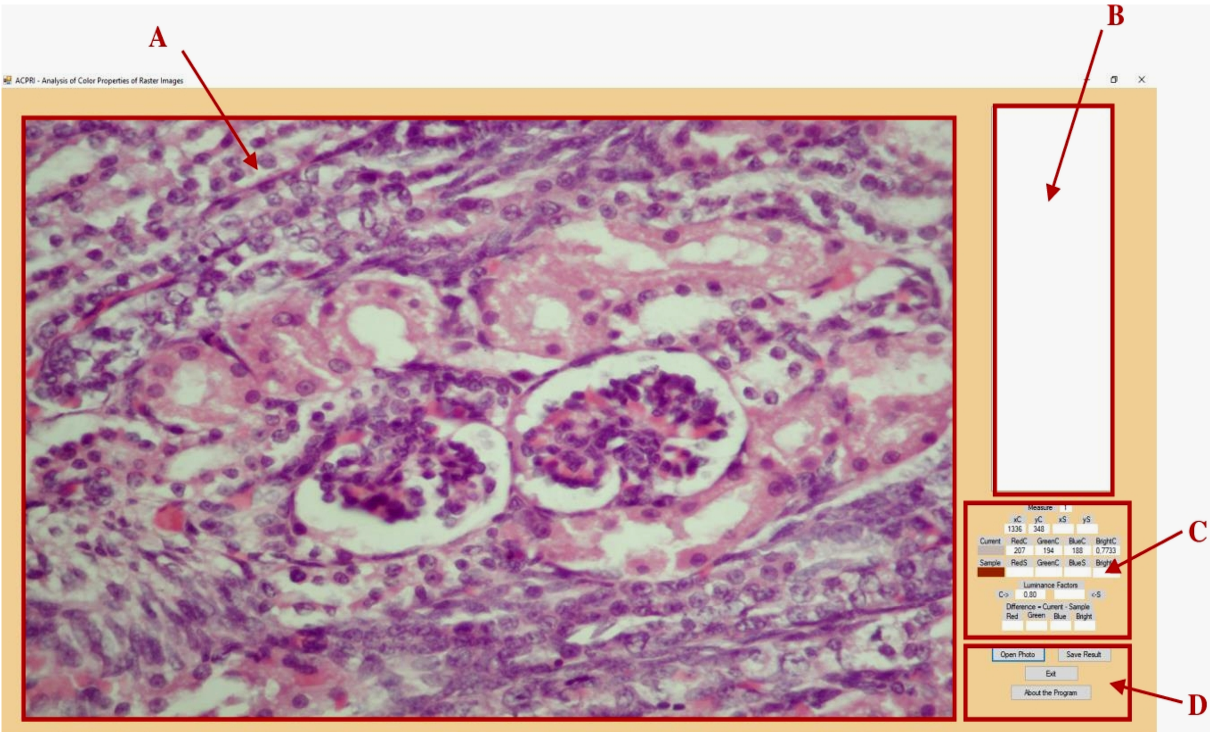

Fig. 1. Basic elements of the user interface of the ACPRI program
(A – a field for displaying a photo;
B – a field of the list of selected pixels;
C – information fields;
D – a field for control buttons)

Information fields «RedC», «GreenC», «BlueC» are values of color components of the current pixel according to the RGB color model, and «BrightC» is the brightness of the current pixel.

Information fields «RedS», «GreenS», «BlueS», «BrightS» are the values of the color components of the sample pixel according to the RGB color model, and «BrightS» is the brightness of the sample pixel.

Information field «Luminance Factor C->» is the brightness of the current pixel in the Lab color model.

Information field «Luminance Factor <-S» shows brightness of the sample pixel in the Lab color model.

Information fields «Difference = Current – Sample: Red, Green, Blue, Bright) are the arithmetic difference between the color components of the current and sample pixel».

The field of control buttons (Fig. 1) contains four buttons with the following functions: (Open Photo) – loads an image from a file; (Save Result) – forms a text file with the analysis results; (Exit) – terminates work with the program; (About the Program) – displays information about the program.

Having formed the list of selected pixels, the user clicks on the «Save Result» button, forms a text file with the results and the statistical analysis. The results file consists of three parts. The first part presents the color parameters of the selected pixels (a number of pixel in the table, the coordinates of the pixel in the photograph, the color components and the brightness value in the RGB and Lab models) (Fig. 3). The second and third parts are statistical characteristics (average, expected value, dispersion, mean square deviation, standard error of the mean) for pixels brightness in the RGB color model (Fig. 4) and Lab (Fig. 5).

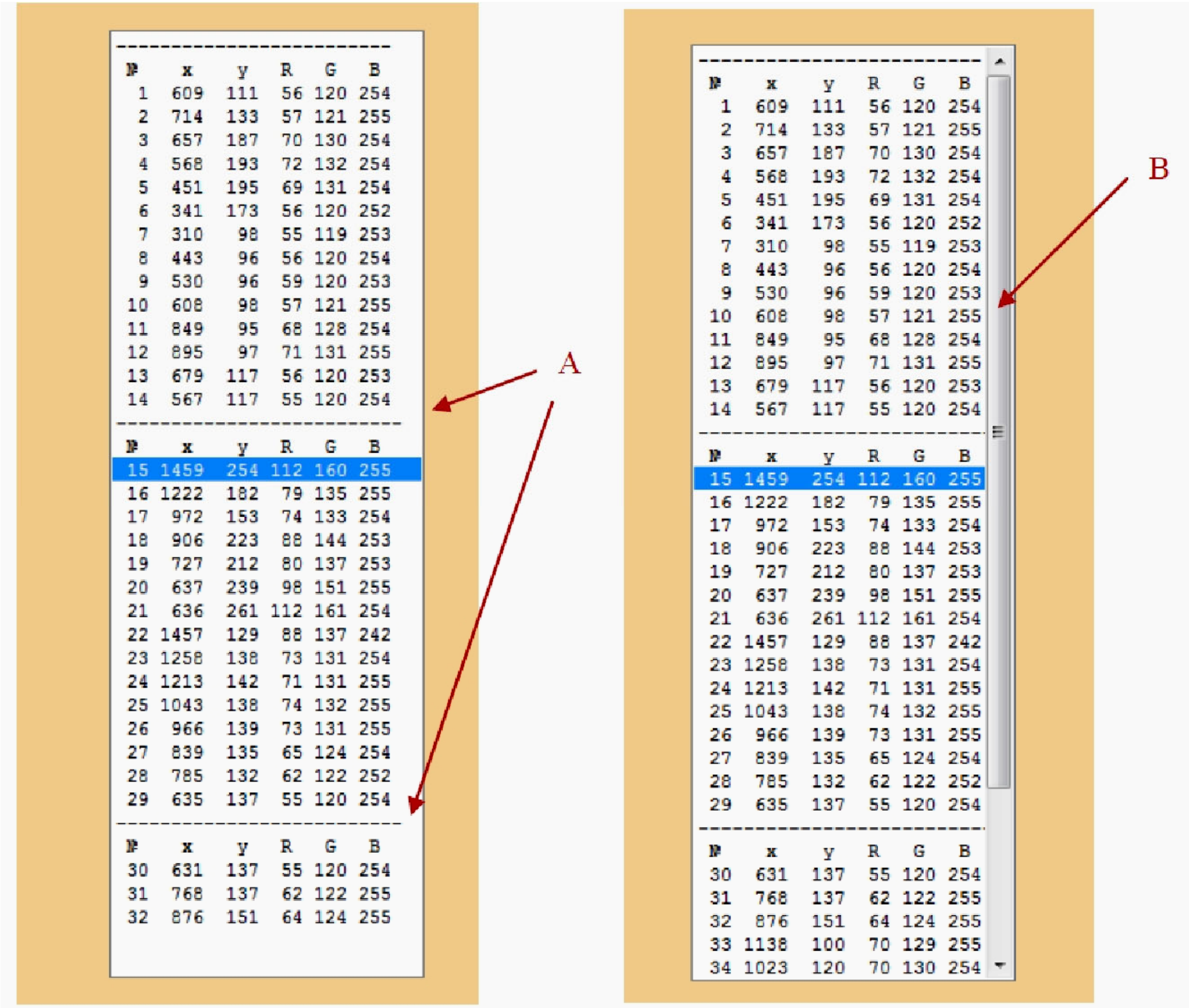

Fig. 2. Field of the list of selected pixels (A – group separators; B – scroll bar)

N	x	y	R	G	B	k	L	a	b
1	437	84	56	123	254	0,4624	89	377	48
2	533	119	54	121	254	0,4554	89	374	50
3	434	168	55	119	253	0,4515	89	374	51
4	286	150	51	117	255	0,4431	90	374	52
5	518	131	53	119	255	0,4501	90	377	51
6	673	120	56	120	254	0,4555	89	374	50
7	622	174	62	125	254	0,4740	89	377	48
8	523	157	56	120	255	0,4559	90	377	51
9	262	72	57	121	253	0,4585	89	374	50
10	554	104	57	121	255	0,4594	90	377	51
11	863	78	71	131	255	0,4988	90	380	45

Fig. 3. Example of displaying information on selected pixels

```
Brightness statistics for the RGB model:
   Average                      0,4852
   Expected value               0,4852
   Dispersion                   0,0005
   Mean square deviation        0,0222
   Standard error of the mean   0,0041
```

Fig. 4. Statistical information for brightness according to RGB model

```
Brightness statistics for the Lab model:
   Average                      0,8969   (89,69)
   Expected value               0,8969   (89,69)
   Dispersion                   0,0000   (0,2140)
   Mean square deviation        0,0046   (0,4626)
   Standard error of the mean   0,0009   (0,0859)
```

Fig. 5. Statistical information for brightness according to Lab model.

Therefore, to solve practical problems when working with digital images of microspecimens, you need to have appropriate methods of digital processing and analysis. Digital image analysis software is constantly in need of improvement to objectify research results. The proposed computer procedure, unlike others [8, 11], which uses one of the digital color models, allows you to analyze the color of each pixel of the photo in RGB and Lab color models, compare colors and brightness of individual pixels, select groups of points and determine statistical characteristics of selected groups. In the photos, the color properties of which are studied using the program "Analysis of color properties of raster images", the characteristic points are clearly visible. This allows computer recognition algorithms to be used to isolate them and to automate their selection, which completely removes the influence of the human factor on the results of the analysis.

Thus, the presented computer program for analysis of digital color images of micropreparations can be a reliable tool in the work of both scientists and physicians who describe and evaluate digital images of biological tissues, along with others that are already widespread and practical [8]. Further improvement and use of digital tools has some prospects depending on the industry in which they are used. The openness of these digital instruments is a reliable way to find the optimal solution to problems related to the standardization of microscopic analysis [12].

CONCLUSIONS

1. In the course of the research, the authors developed a modern computer program «Analysis of color properties of raster images". This program allows you to analyze the color of each individual pixel of a photograph in RGB and Lab color models, comparing the colors and brightness of individual pixels, selecting groups of points and determining statistical characteristics of them.
2. Characteristic points are well distinguishable in the photographs, studied with the program «Analysis of color properties of raster images». This makes it possible to select and automate these properties, using computer recognition algorithms, completely removing human factor's influence on the analysis results.
3. The computer program «Analysis of color properties of raster images» is of significant scientific and practical interest for specialists both in the field of morphology (pathologists, forensic experts, etc.), and in the field of other biomedical disciplines.

Contributors:

Ivanova M.D. – writing – review and editing;

Myroshnychenko M.S. – methodology, writing – original draft, project administration;

Khara G.I. – conceptualization, software;

Arseniev O.V. – data curation, funding acquisition;

Olkhovsky V.O. – validation, visualization;

Grygorian E.K. – investigation;

Fedulenkova Yu.Ya. – resources, supervision;

Kozlov S.V. – formal analysis.

Funding. This research received no external funding.

Conflict of interests. The authors declare no conflict of interest.

REFERENCES

1. Volkov V. [To the assessment of indicators in morphological medicobiological researches: correction of approach to new complex statistical algorithm]. Bulletin of science and practice. 2016;6:75-84. Russian.
2. Gorbunova EV, Chertov AN. [Colorimetry of radiation sources: textbook]. SPb: Universitet ITMO; 2015. p. 126. Russian.
3. Erofeev SV, Shishkin YY, Fedorova AS. [About the technology of image analysis as a means of increasing the objectivity and reliability of forensic examinations]. Russian Journal of Forensic Medicine. 2017;3(2):17-23. Russian. doi: https://doi.org/10.19048/2411-8729-2017-3-2-17-23
4. Kitrar LA, Lipkind TM, Ostapkovich GV. [Quantification of qualitative variables in business surveys]. Voprosy statistiki. 2018;25(4):49-63. Russian.
5. Kovalskiy BM, Dudiak VO, Zanko NV, Pysanchyn NS. [Interaction of basic concepts of colour theory with colour perpoduction in modern digital systems]. Printing and publishing. 2018;1(75):19-30. Ukrainian. doi: https://doi.org/10.32403/0554-4866-2018-1-75-19-30
6. Review Bagriy MM, Dibrova VA, Popadynets OG, Grischuk MI. [Methods of morphological research]. Bagriy MM, Dibrova VA, editors. Vinnitsa: New book; 2016. p. 328. Ukrainian.
7. Myroshnychenko MS, Dyadyk OO, Olkhovsky VO, Grygorian EK. [Digital technologies and their diagnostic value in pathological anatomy and forensic medicine: current state of the problem]. Collection of scientific article of the 3 International scientific and practical conference «Informational systems and technologies in medicine» (November 26-27, 2020, Kharkiv). Kharkiv: HNURE; 2019. p. 59-60. Ukrainian.
8. Poslavska OV. [Methodology for the use of software for the analysis of digital micrographs on the base of pathomorphology course in order to increase the professional level of students and scientists]. Morphologia. 2015;9(3):122-6. Ukrainian. doi: https://doi.org/10.26641/1997-9665.2015.3.122-126
9. Starovoitov VV, Golub YuI. [Digital images: from acquisition to processing]. Minsk: OIPI NAN Belarusi; 2014. p. 202. Russian.
10. Dhingra V, Juglan S. Importance of medico legal expert at scene of crime related to death. Journal of forensic sciences and criminal investigation. 2017;6(1). doi: https://doi.org/10.19080/JFSCI.2017.06.555682
11. Gurcan MN, Boucheron LE, Can A, Madabhushi A, Rajpoot NM, Yener B. Histopathological image analysis: a review. IEEE Rev Biomed Eng. 2009;2:147-71. doi: https://doi.org/10.1109/RBME.2009.20348655.
12. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ ecosystem: an open platform for biomedical image analysis. Mol Reprod Dev. 2015;1-12.
13. Silva LFF, Saldiva PHN, Alves VAF. History and prospects of pathology in medicine. Revista de Medicina. 2016;95(2):68-72. doi: https://doi.org/10.11606/issn.1679-9836.v95ispe2p68-72
14. Windows Presentation Foundation. [Internet]. Available from: https://ru.wikipedia.org/wiki/Windows_Presentation_Foundation

Стаття надійшла до редакції
24.09.2021