Penerbit Akademia Baru

Journal of Advanced Research Design

Journal homepage:
https://akademiabaru.com/submit/index.php/ard
ISSN: 2289-7984

Automatic Colour Staining on Slide Histopathology Images using RGB Image Processing

Wan Azani Mustafa[1,2,*], Muhammad Afiq Zharfan Ishak[1], Khalis Khiruddin[2], Khairul Shakir Rahman[3] and Moh. Khairudin[4]

[1] Faculty of Electrical Engineering & Technology, Universiti Malaysia Perlis, UniCITI Alam Campus, Sungai Chuchuh, 02100 Padang Besar, Perlis, Malaysia
[2] Advanced Computing (AdvComp), Centre of Excellence (CoE), Universiti Malaysia Perlis (UniMAP), Campus Pauh Putra, 02600 Arau, Perlis, Malaysia
[3] Department of Pathology, Hospital Tuanku Fauziah, 01000 Kangar, Perlis, Malaysia
[4] Department of Electrical Engineering, Universitas Negeri Yogyakarta, Karangmalang, Yogyakarta 55281, Indonesia

ARTICLE INFO

Article history:
Received 7 February 2025
Received in revised form 7 March 2025
Accepted 30 June 2025
Available online 20 July 2025

Keywords:
Automatic staining; artificial intelligent; colour cell; histopathology; image processing

ABSTRACT

Nowadays, staining on slide histopathology images is widely utilized in medicine. The histopathology analysis is conducted manually by the pathologist, including tissue structure, distribution of cells in tissues and others. However, since this assessment is generally performed visually by pathologists, it can suffer from significant inter-observer variability. To overcome this difficulty, automatic staining on slide histopathology using assisted image processing provided a more quantitative diagnosis of cells. This is a step in preparing automatic staining on slide histopathology images using image processing. Firstly, convert the original picture from the hospital to a grayscale picture since the original picture from the hospital is overly bright using the ImageJ application. ImageJ converts the original picture from the hospital to grayscale by setting the image to 32 bits and adjusting the brightness and contrast. Then, after obtaining the grayscale picture, the second step is converting the grayscale to an RGB colour. This step is implemented using the Matrix Laboratory (MATLAB) coding application and then the comparison result of the grayscale picture and the final picture from manual staining is obtained. For the result analysis, this research used an online application to examine the colour similarity between final manual staining and automatic staining using MATLAB. The similarity of colour staining by MATLAB exceeds 50% for all samples compared to manual staining. In conclusion, the application of automatic staining on slide histopathology using an image processing technique is a much better method to classify each cell.

1. Introduction

Histopathology is the study of disease certainty using microscope slide staining. With this slide staining technique, one can observe the structure of the tissue or cell more accurately [1]. Several methods are employed to study the characteristics of a tissue or cell. In addition, using different

* *Corresponding author*
E-mail address: wanazani@unimap.edu.my

https://doi.org/10.37934/ard.138.1.113

stains allows the tissue or cell to be examined more easily. These histological studies are used in several situations, such as forensic investigation, autopsy and diagnosis in education [2]. In addition, these histological studies are utilized in medicine to treat diseased tissues.

Staining histopathology images on slides is prevalent in medicine nowadays [3]. However, with the old technique, manually staining cells is inappropriate [4]. With this research, all the work is automatic to obtain the results. Furthermore, when manually staining on slide histopathology image, the doctor takes a long time to detect the staining cell since manual staining requires some steps to be followed, such as fixing, processing, embedding the sample in wax, sectioning and staining [5]. Notably, every stage must be adhered to precisely and the entire process requires significant time to yield results. Other than that, the old technique can slow down staff work since each step above cannot be completed in one day [6]. Additionally, even if the result is obtained, it could be incorrect due to a human error mistake. Hence, the outcome of this problem is that the pathologist may take longer to diagnose the cell and the pathologist may misdiagnose the condition of the cell [7].

1.1 Cell

The human body contains a trillion cells and cells communicate with each other to create a solid. In the human body, cells build tissues, tissues build organs and organs work together to keep the body alive [8]. This part will explain cells, such as inside cells, as displayed in Figure 1, cell division and types of cells in Figure 2. Remarkably, each cell looks different and has a different role in the body [9]. Among those inside the cell is the nucleus, such as the cell headquarters, which then contains most of the cell's deoxyribonucleic acid (DNA) and plasma membrane to ensure each cell separates from other cells. Meanwhile, the cytoplasm is the interior of the cell that surrounds the nucleus. At the same time, the cytoskeleton forms the scaffolding within the cytoplasm of the human body cell and the endoplasmic reticulum processes molecules within the cell and helps transfer them to their final destinations. Moreover, the Golgi apparatus is one of the processes through which the molecules form the endoplasmic reticulum to the Golgi apparatus. Additionally, mitochondria are the powerhouse of the cell and ribosomes are the nucleus that transcribes segments of DNA into Ribonucleic acid (RNA) [10].

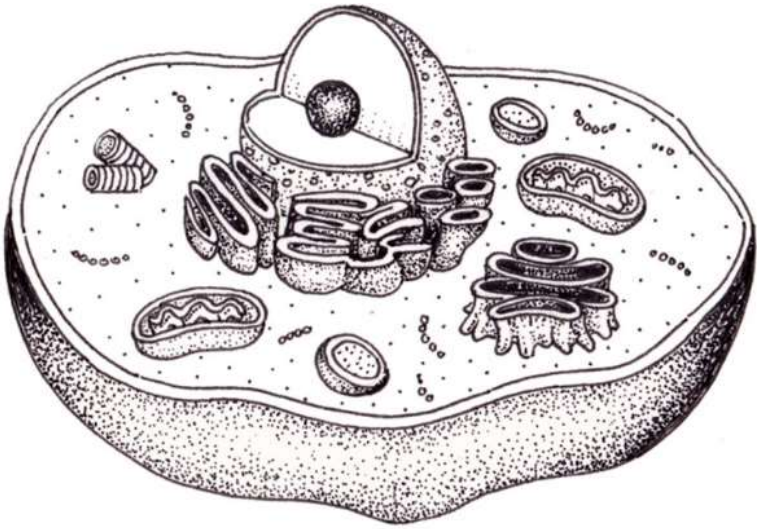


Fig. 1. Cell inside the human body [11]

In the human body, more than 200 different types of cells are present. Nevertheless, this part explains a small selection of types of cells in the human body. Firstly, bone cells. This cell is separated

into three parts: osteoclasts, osteoblasts and osteocytes [12]. Next are the blood cells. Red blood cells carry oxygen around the human body [13], while white blood cells are part of the immune system [14]. In addition, platelets help blood clot to prevent blood loss after injury. This is followed by muscle cells. This cell is vital for a range of functions, including movement, support and internal function [15]. Then, the fat cells. This cell contains stored fat used for the body as energy [16]. Lastly are the nerve cells. This cell communicates with the entire system body [17].

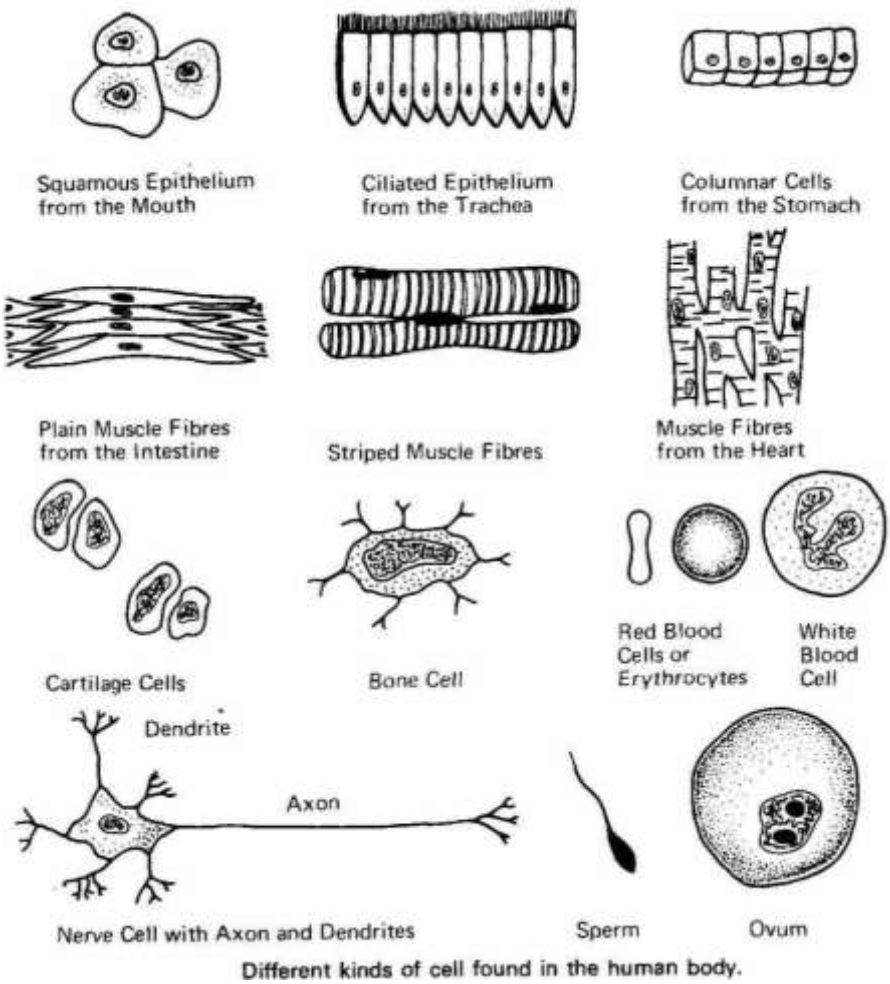


Fig. 2. Cell types in the human body [18]

1.2 Staining

Important tissue features are stained and tissue contrast is also improved. While a different stain dye used in histology offers the cell's nucleus a pink stain, hematoxylin, a fundamental dye typically employed in this technique, offers the nucleus a bluish colour. Notably, various staining methods are available for specific cells and components [19]. In order to identify sick, tumorous or other pathological cells, staining is a commonly employed medical procedure. Staining involves applying a dye colour to the rear and front border of sample tissues [20]. In biological research, staining is used to identify cells and to designate proteins, nucleic acids or gel electrophoresis for microscopic study. In essence, differential staining, double staining and other multiple staining techniques may be employed under certain circumstances [21].

The evolution of staining suggests that the use of histological methods for disease diagnosis is a comparatively recent development. Early pathologists and surgeons used historical staining methods

from the seventeen-year-old scientist Leeuwenhoek, who significantly contributed to histology by colouring tissues with Madder, indigo and saffron and studying them with crude microscopes. These early researchers employed microanatomy to distinguish between the structures of normal plant and animal cells as well as to infer a link between cellular differences [22]. Later, different methods that preserved tissues in their original state prior to staining were developed to improve the detailed investigation of cell structure. When Joseph Von Gerlach successfully stained cerebellar cells in 1858 using ammoniacal carmine, he was recognized as the father of microscopical staining [22].

Early histologists prepared tissues for microscopic examinations using widely accessible chemicals; these laboratory chemicals included potassium dichromate, alcohol and mercuric chloride to harden cellular tissues [23]. Accordingly, these fixatives and staining agents were brilliant and throughout time, coloured staining agents were created that are still useful in modern laboratory staining procedures. The trichrome stain, which is used in liver and kidney biopsies, as well as the silver nitrate stain, which is utilized in other organisms, are two examples of these brilliant-coloured stains that are still in use today [24]. The improvement in microscope technology and the founding of histologic stains (aniline dye) in 1856 in Germany, which manufactures various novel histological stains, both significantly impacted the development of histologic stains [25]. At the same time, research and understanding of the human body's tissues and anatomy grew and this understanding was practiced in new histological procedures for the analysis of sick tissue [26]. Following the eighteenth century, many hospitals started hiring doctors, pathologists and surgeons to care for surgical concerns. This generation of pathologists is responsible for developing intraoperative staining methods for frozen tissue sections by modifying a unique staining method from histopathology. The paraffin infiltration staining method was developed at this period. This accomplishment allowed researchers to study both benign and malignant tumours [27], which led to the discovery of a bacterium as the disease's etiological agent in the nineteenth century.

In order to distinguish between various bacterial species, a Danish scientist called Hans Christian Gram developed the Gram staining technique in 1875. In order to differentiate the kind of bacterial infection and to make the germs visible on certain lung tissues during inspection, Gram developed the staining process while working at the municipal morgue with his co-workers. Even though some bacterial species were discovered to be inappropriate for this approach, it is still in use today and reasonably competes with contemporary molecular histology techniques [28]. Despite significant advances in the evolution of histological staining techniques, several critical gaps remain. While historically significant, traditional staining methods often suffer from issues related to variability, lack of standardization and incompatibility with modern digital and automated analysis techniques. Furthermore, current practices do not fully address the need for faster, more reproducible and machine-compatible staining protocols, particularly in the context of high-throughput and Artificial Intelligence (AI)-driven diagnostics. Correspondingly, these challenges highlight the necessity for developing or adapting staining approaches optimized for contemporary medical and research applications.

This study aims to develop a fully automated staining technique that transfers colour information from a manually stained reference image to a grayscale target image using luminance similarity in the YCbCr colour space. By separating the luminance (structural details) from the chrominance (colour components), the proposed method ensures that the tissue morphology is preserved during the colourization process. This approach addresses key limitations of traditional manual staining, such as variability, labour intensity, and time consumption, while also enabling standardized colourization suitable for automated digital pathology systems. To the best of our knowledge, this is one of the first frameworks that applies luminance-guided colour transfer specifically for histological

staining simulation. It holds the potential to facilitate the integration of virtual staining into diagnostic workflows more efficiently.

The study has two main objectives:

1. To develop an automatic staining method for histopathology slide images using MATLAB software.

2. To evaluate the efficiency of the proposed method by comparing it with traditional manual staining.

For this purpose, histopathological data were collected from Hospital Tuanku Fauziah, Kangar. A total of 12 sample images were used, including the original cell images before staining and their manually stained counterparts. Before applying the automated staining method, the original images were first converted to grayscale using the ImageJ application. MATLAB (version R2020a) was then used to apply colour to these grayscale images. Finally, an analysis was conducted to evaluate the colour similarity between the automatically stained images and the manually stained images. This analysis was performed using the online tool IMGonline.com.ua, with a similarity threshold set at a minimum of 50%.

2. Methodology

The method of conducting this research involves a variety of aspects to consider. One of the major aspects is the technical constraints and limitations desired to develop the project. Figure 3 illustrates the block diagram of this study. The material used for this project includes the ImageJ application, MATLAB and online application. Meanwhile, the method used for this research is mapping between final manual staining and the grayscale picture of the cell.

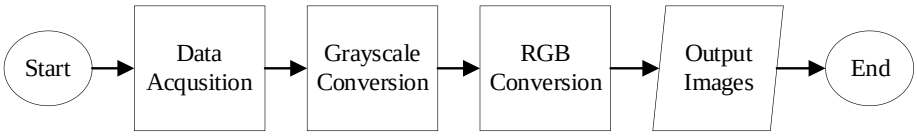


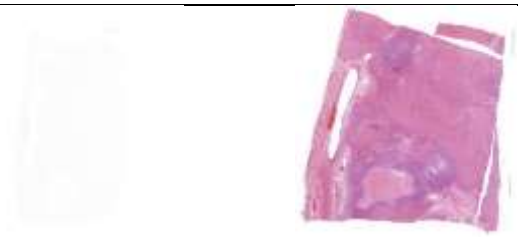
Fig. 3. Block diagram of study

2.1 Data Acquisition

Histopathology data was collected from Hospital Tuanku Fauziah, Kangar. The dataset consists of 12 sample images, including original cell images before staining and manually stained images. The original image and manual staining result can be observed in Table 1.

Table 1
Sample of original cell images and manually staining results

No.	Before staining	After manual staining
1.		

2.	
3.	
4.	

2.2 ImageJ Application

ImageJ is an open-source image processing software developed by the National Institutes of Health (NIH) and widely used in scientific and medical imaging applications. It provides functionalities such as visualization, annotation, calibration and quantitative analysis of images across various formats. In this study, ImageJ was specifically utilized for grayscale conversion as part of the initial preprocessing stage.

As depicted in Figure 3, the workflow begins with image data acquisition, followed by grayscale conversion performed using ImageJ. Medical images obtained from hospital datasets often exhibit low contrast and high brightness, which can obscure structural details and compromise further analysis. Building on this, converting these images to grayscale reduces visual noise and enhances relevant features by eliminating unnecessary colour information. Hence, this step is crucial for improving the accuracy of subsequent processing.

Following the grayscale conversion depicted in Table 2, the images were exported for further manipulation using MATLAB, which included RGB reconstruction and preparation of output datasets for analysis. The use of ImageJ at this stage ensured consistent grayscale conversion and standardized the input for downstream image processing. It can also be used to display, annotate, edit, calibrate, measure, analyse, process, print and save raster row and column image data. Furthermore, it supports most common raster image formats as well as raw data files in text format, such as those from spreadsheets. Therefore, this research utilized ImageJ to convert original hospital data images into grayscale. Additionally, hospital image data tends to have low contrast and excessive brightness, making grayscale conversion necessary for better analysis. For this reason, ImageJ was preferred for this project.

Table 2
Comparison of original picture cell with grayscale picture

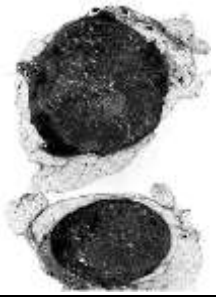
No.	Picture before dye (original from hospital)	Grayscale picture using ImageJ
1		
2		
3		

Table 2 above compares the difference between the original pictures of the cell before the dye and the grayscale pictures after using the ImageJ application. Subsequently, the picture has to be converted to a grayscale picture since the original picture is overly bright. Then, grayscale pictures are converted to RGB colour in MATLAB.

2.3 Automated Staining Algorithm

Algorithm 1 outlines a fully automated staining method that transfers colour from a reference colour image to a target grayscale image by leveraging luminance similarity in the YCbCr colour space. The grayscale input image I_g and the reference colour image IcI_cIc are both transformed into YCbCr space, separating luminance (Y) from chrominance (Cb and Cr). This separation enables targeted manipulation of colour without altering the structural content of the input.

Algorithm 1. The conversion of the grayscale image to RGB using YCbCr color space.

```
Automated_Staining(img1, img2)
  Input: img1 (grayscale image), img2 (color image)
  Output: R (colorized image)

  Read img1, img2
  If img1 is not grayscale then
    Convert img1 to grayscale
  End if
  If img2 is not a 3-channel color image then
    Error: img2 must be a color image
  End if

  nspace1 ← rgb2ycbcr(img2)
  nspace2 ← rgb2ycbcr(img1)

  Normalize Y channels of nspace1 and nspace2 to [0, 255]

  For each pixel (i, j) in img1 do
    Find (r, c) minimizing |Y_img2(r,c) - Y_img1(i,j)|
    Assign:
      Cb_output(i,j) ← Cb_img2(r,c)
      Cr_output(i,j) ← Cr_img2(r,c)
      Y_output(i,j) ← Y_img1(i,j)
  End for

  R ← ycbcr2rgb(Y_output, Cb_output, Cr_output)
  Return R
End Algorithm
```

To ensure compatibility between the images, the luminance channels of both images are normalized to the range [0,255]. For an image I, its normalized luminance channel Y_{norm} is computed as:

$$Y_{norm}(x,y) = \frac{Y(x,y)-Y_{min}}{Y_{max}-Y_{min}} \times 255 \quad (1)$$

where Y_{min} and Y_{max} denote the minimum and maximum luminance values in the image, respectively.

For each pixel (x,y) in the grayscale image I_g, the algorithm identifies the pixel (r,s) in the reference image I_c whose luminance value is closest in absolute difference:

$$(r,s) = \arg\min_{(i,j)} \left| Y^{c}_{norm}(i,j) - Y^{g}_{norm}(x,y) \right| \quad (2)$$

Once a match is determined, the chrominance components from the reference image at the location (r, s), denoted as $Cb^c(r, s)$ and $Cr^c(r, s)$, are transferred to the target pixel (x, y) while retaining the original luminance value:

$$Cb^{out}(x, y) = Cb^c(r, s), Cr^{out}(x, y) = Cr^c(r, s), Y^{out}(x, y) = Y^g(x, y) \quad (3)$$

This results in a reconstructed image in the YCbCr space:

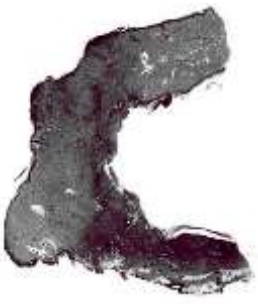
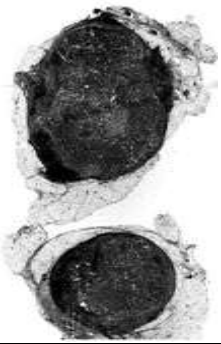
$$I^{out}_{YCbCr}(x,y)=[Y^{out}(x,y), Cb^{out}(x,y), Cr^{out}(x,y)] \quad (4)$$

Finally, the image is converted back to RGB space:

$$R(x, y) = YCbCr2RGB(I^{out}_{YCbCr}(x, y)) \quad (5)$$

This process yields a stained image that preserves the grayscale structure while adopting the chromatic characteristics of the reference image. The method is efficient, non-parametric and visually effective for applications such as medical image enhancement and synthetic staining in histopathology.

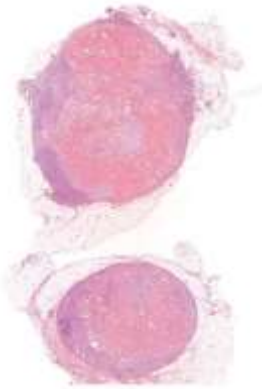
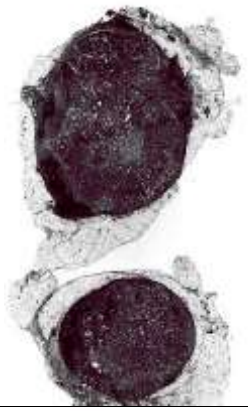
Table 3
Comparison of grayscale and RGB colour

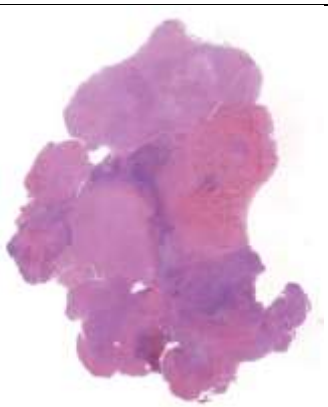
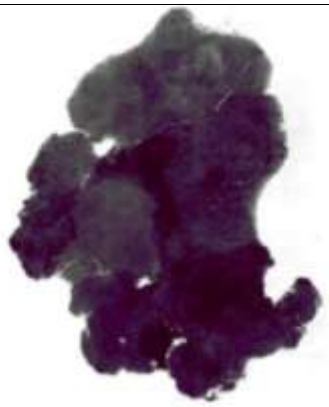
No	Grayscale picture	RGB colour
1		
2		
3		

3. Results and Discussion

The research outcome is the progress of the design and development of the desired system. The analysis image has been performed to identify the performance in the colour similarity between manual staining and automatic staining using MATLAB. The analysis result will be examined based on the colour similarity between the manual staining image and automatic staining using MATLAB. To yield a better result, the colour similarity must exceed 50%. From Table 4 below, the similarity value exceeds 50%. The colour similarity check is conducted using an online app, IMGonline.com.ua.

Table 4
Comparison results between manual staining and automatic staining

No.	Output Manual Staining Image	Automatic Staining Image using MATLAB	Similarity
1			58.62%
2			54.49%
3			67.89%

4			66.58%
5			59.95%

Based on this research, the first step is to collect data from Hospital Tuanku Fauziah, Kangar. Next, all the data provided is converted to grayscale since the original hospital images are excessively bright. The ImageJ application is used for this conversion, requiring the individual manual processing of each dataset.

To begin, open the image in ImageJ and select the 32-bit option for the picture. Then, navigate to the "Adjust" menu and choose "Brightness and Contrast." This application allows for adjustments either automatically or manually. Once the desired settings are applied, save the image as a grayscale picture. This process is repeated for all datasets to obtain the complete set of grayscale images.

Subsequently, convert grayscale to RGB colour using MATLAB. Coding is used to obtain the result for this part. The coding for this project is divided into two parts. The first part includes writing the function grayscale to RGB colour. This part also writes the coding with regard to conversion, normalization and luminance comparison. In particular, the conversion is about converting a grayscale image to RGB. Following this, normalization is the output image manual staining from Hospital Tuanku Fauziah, Kangar. Then, luminance comparison refers to the comparison between the grayscale image and the output manual staining image, resulting in a new image. Conversely, the second part pertains to writing the callback function in part one, then including the location grayscale and final picture in JPG. Then, after both pictures are compared, a new picture in RGB colour is obtained.

4. Conclusions

In conclusion, the proposed automatic method for staining on slide histopathology images has been completed and achieved the first objective of this research. The method in this research is mapping by MATLAB coding. This method maps the final result of the manual staining provided by

Hospital Tuanku Fauziah, Kangar, with a grayscale image after converting the original image cell provided by Hospital Tuanku Fauziah, Kangar, using ImageJ software, resulting in the final image. Then, to analyse the efficiency of the proposed method, a comparison to the manual method was completed, accomplishing the second objective of this project. For this part, the analysis efficiency is based on the colour similarity compared between the final results of manual and automatic staining using MATLAB. For better results, the colour similarity must exceed 50%. Finally, the analysis results reveal that the performance of automatic staining is more efficient than manual staining. That is, automatic staining saves more time and costs less than manual staining. Therefore, it can be concluded that this project is a good alternative to producing clean energy and can benefit the community with the low cost of checking cells. In addition, this research is safe from the point of view of health, safety and law, as it does not involve any direct contact with the body to collect cells.

Acknowledgement
This research was supported by funding from the Ministry of Higher Education (MOHE) Malaysia under the fundamental research grant scheme (FRGS/1/2021/SKK0/UNIMAP/02/1).

References

[1] Srinivasan, M. R., A. Sabarinathan, A. Geetha, K. Shalini and M. Sowmiya. "A comparative study on staining techniques for vaginal exfoliative cytology of rat." *J Pharmacol Clin Res* 3, no. 3 (2017): 1-4. https://doi.org/10.19080/JPCR.2017.03.555615

[2] Dorovskikh, Svetlana I., Evgeniia S. Vikulova, Elena V. Chepeleva, Maria B. Vasilieva, Dmitriy A. Nasimov, Eugene A. Maksimovskii, Alphiya R. Tsygankova, Tamara V. Basova, David S. Sergeevichev and Natalya B. Morozova. "Noble metals for modern implant materials: MOCVD of film structures and cytotoxical, antibacterial and histological studies." *Biomedicines* 9, no. 8 (2021): 851. https://doi.org/10.3390/biomedicines9080851

[3] Schüürmann, M., M. M. Stecher, U. Paasch, J. C. Simon and S. Grunewald. "Evaluation of digital staining for ex vivo confocal laser scanning microscopy." *Journal of the European Academy of Dermatology and Venereology* 34, no. 7 (2020): 1496-1499. https://doi.org/10.1111/jdv.16085

[4] Czubaszek, Magdalena, Katarzyna Andraszek, Dorota Banaszewska and Renata Walczak-Jędrzejowska. "The effect of the staining technique on morphological and morphometric parameters of boar sperm." *PloS one* 14, no. 3 (2019): e0214243. https://doi.org/10.1371/journal.pone.0214243

[5] Wemmert, Cédric, Jonathan Weber, Friedrich Feuerhake and Germain Forestier. "Deep learning for histopathological image analysis." *Deep Learning for Biomedical Data Analysis: Techniques, Approaches and Applications* (2021): 153-169. https://doi.org/10.1007/978-3-030-71676-9_7

[6] Đorđević, Marija, Maja Životić, Sanja Radojević Škodrić, Jelena Nešović Ostojić, Jasmina Marković Lipkovski, Jelena Filipović, Sanja Ćirović, Sanjin Kovačević and Duško Dunđerović. "Effects of automation on sustainability of immunohistochemistry laboratory." In *Healthcare*, vol. 9, no. 7, p. 866. MDPI, 2021. https://doi.org/10.3390/healthcare9070866

[7] Raihan, Md Johir and Abdullah-Al Nahid. "Malaria cell image classification by explainable artificial intelligence." *Health and Technology* 12, no. 1 (2022): 47-58. https://doi.org/10.1007/s12553-021-00620-z

[8] Zebrowska, Aneta, Agata Skowronek, Anna Wojakowska, Piotr Widlak and Monika Pietrowska. "Metabolome of exosomes: Focus on vesicles released by cancer cells and present in human body fluids." *International journal of molecular sciences* 20, no. 14 (2019): 3461. https://doi.org/10.3390/ijms20143461

[9] Beenken andrew. "Endocytosis begins inside the cell." *Journal of the American Society of Nephrology* 33, no. 4 (2022): 661-662. https://doi.org/10.1681/ASN.2022020155

[10] Paunovska, Kalina, David Loughrey and James E. Dahlman. "Drug delivery systems for RNA therapeutics." *Nature Reviews Genetics* 23, no. 5 (2022): 265-280. https://doi.org/10.1038/s41576-021-00439-4

[11] Aryee, Patrick. *30 Animals that Made Us Smarter: Stories of the Natural World that Inspired Human Ingenuity*. Island Press, 2022.

[12] Ma, Xiaoli, Peihong Su, Chong Yin, Xiao Lin, Xue Wang, Yongguang Gao, Suryaji Patil *et al.,* "The roles of FoxO transcription factors in regulation of bone cells function." *International journal of molecular sciences* 21, no. 3 (2020): 692. https://doi.org/10.3390/ijms21030692

[13] Pretini, Virginia, Mischa H. Koenen, Lars Kaestner, Marcel HAM Fens, Raymond M. Schiffelers, Marije Bartels and Richard Van Wijk. "Red blood cells: chasing interactions." *Frontiers in physiology* 10 (2019): 945. https://doi.org/10.3389/fphys.2019.00945
[14] Nassar, Mariam, Minh Doan andrew Filby, Olaf Wolkenhauer, Darin K. Fogg, Justyna Piasecka, Catherine A. Thornton *et al.,* "Label-free identification of white blood cells using machine learning." *Cytometry Part A* 95, no. 8 (2019): 836-842. https://doi.org/10.1002/cyto.a.23794
[15] Shi, Jia, Yi Yang, Anying Cheng, Gang Xu and Fan He. "Metabolism of vascular smooth muscle cells in vascular diseases." *American Journal of Physiology-Heart and Circulatory Physiology* (2020). https://doi.org/10.1152/ajpheart.00220.2020
[16] Shah, Mickey, Richard L. George, M. Michelle Evancho-Chapman and Ge Zhang. "Current challenges in dedifferentiated fat cells research." *Organogenesis* 12, no. 3 (2016): 119-127. https://doi.org/10.1080/15476278.2016.1197461
[17] Schafer, Simon T. and Fred H. Gage. "Nerve cells from the brain invade prostate tumours." (2019): 637-638. https://doi.org/10.1038/d41586-019-01461-7
[18] Alberts, Bruce, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter. "Molecular Biology of the Cell. 2002 Garland Science." *New York*.
[19] Ribatti, Domenico. "The staining of mast cells: a historical overview." *International archives of allergy and immunology* 176, no. 1 (2018): 55-60. https://doi.org/10.1159/000487538
[20] Rick, Jonathan, Arman Jahangiri, Patrick M. Flanigan, Ankush Chandra, Sandeep Kunwar, Lewis Blevins and Manish K. Aghi. "Growth hormone and prolactin-staining tumors causing acromegaly: a retrospective review of clinical presentations and surgical outcomes." *Journal of neurosurgery* 131, no. 1 (2018): 147-153. https://doi.org/10.3171/2018.4.JNS18230
[21] Nygate, Yoav N., Mattan Levi, Simcha K. Mirsky, Nir A. Turko, Moran Rubin, Itay Barnea, Gili Dardikman-Yoffe, Miki Haifler, Alon Shalev and Natan T. Shaked. "Holographic virtual staining of individual biological cells." *Proceedings of the National Academy of Sciences* 117, no. 17 (2020): 9223-9231. https://doi.org/10.1073/pnas.1919569117
[22] Bracegirdle, B. "The history of staining." In *Conn's Biological Stains*, pp. 15-21. Taylor & Francis, 2020. https://doi.org/10.1201/9781003076841-2
[23] Alturkistani, Hani A., Faris M. Tashkandi and Zuhair M. Mohammedsaleh. "Histological stains: a literature review and case study." *Global journal of health science* 8, no. 3 (2015): 72. https://doi.org/10.5539/gjhs.v8n3p72
[24] Sam-Yellowe, Tobili Y., Troy A. Getty, Kush Addepalli, Aidan M. Walsh, Alberto R. Williams-Medina, Hisashi Fujioka and John W. Peterson. "Novel life cycle stages of Colpodella sp.(Apicomplexa) identified using Sam-Yellowe's trichrome stains and confocal and electron microscopy." *International Microbiology* (2022): 1-10. https://doi.org/10.1007/s10123-021-00175-z
[25] Jayapandian, Catherine P., Yijiang Chen andrew R. Janowczyk, Matthew B. Palmer, Clarissa A. Cassol, Miroslav Sekulic, Jeffrey B. Hodgin *et al.,* "Development and evaluation of deep learning–based segmentation of histologic structures in the kidney cortex with multiple histologic stains." *Kidney international* 99, no. 1 (2021): 86-101. https://doi.org/10.1016/j.kint.2020.07.044
[26] Kandel, Eric R. "Two Opposing Views Have Been Advanced on the Relationship Between Brain and Behavior." *Principles of neural science* (2000): 5-6.
[27] Marhana, Isnin Anang, Kadek Widianiti and Etty Hary Kusumastuti. "Conformity of Fine Needle Aspiration Biopsy (FNAB) and Core Needle Biopsy (CNB) in peripheral lung tumor patients: A cross-sectional study." *Annals of Medicine and Surgery* 75 (2022): 103423. https://doi.org/10.1016/j.amsu.2022.103423
[28] Wu, Kong-Yang and Tong-Xiang Yang. "A novel improved gram staining method based on the capillary tube." *Polish Journal of Microbiology* 69, no. 4 (2020): 503. https://doi.org/10.33073/pjm-2020-043