ORIGINAL COMMUNICATION

Anatomy Journal of Africa. 2023. Vol 12 (1): 2252-2262

PREPARATION OF HISTOLOGY SLIDES AND PHOTOMICROGRAPHS: INDISPENSABLE TECHNIQUES IN ANATOMIC EDUCATION.

Ubi Essien Isaac[1], Emmanuella Oyo-Ita[2], Nancy Paulina Igwe[3], Eze Lazarus Ije[3]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Cross River University of Technology, Nigeria

[2]Department of Biochemistry, Faculty of Science, Cross River University of Technology, Calabar, Nigeria

[3]Department of Anatomy, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Nigeria

Corresponding Author: Dr Ubi E. Isaac, E-mail: isaacubi@unicross.edu.ng. ORCID: 0000-0003-4577-6278

ABSTRACT

The relevance of histology slides in microscopy cannot be overemphasized. However, a major challenge to many scientists in the field stems from paucity of published data to nearly lack of access to a comprehensive technical guide to prepare tissue samples as needed for practical demonstrations, reviews or diagnostic purposes. The current research project was carried out to produce histology glass slides and photomicrographs, as well as to document the step-by-step procedures for model by concerned practitioners and scientists. The standard procedures for paraffin method of histological preparation were employed. This involves sample fixation, dehydration, clearing, impregnation, embedding, section-cutting, staining and mounting. Harris hematoxylin and eosin were used for section staining. Tissue specimens were sourced from laboratory rats (*Rattus norvegicus*). Other materials used included reagents- formalin, alcohol, xylene, distyrene plasticizer xylene (DPX) as well as instruments/equipment- embedding mould, water bath, oven and rotary microtome. Photomicrographs were produced using digital camera attached to light microscope that is connected to a computer interface. 500 pieces set of slides comprising 20 different organ specimens was produced as samples, with each specimen mounted on 25 glass slides of either longitudinal or transverse sections. 40 digital images taken at low and high magnifications were also generated from the various specimens and subsequently stored on a disk. This project occupies a special place in practice due to the clarity about the techniques and the good quality of the slide series obtained which both contribute to knowledge.

Keywords: tissue processing, embedding, microtome, photomicrographs, microscopy

DOI: https://dx.doi.org/10.4314/aja.v12i1.1

INTRODUCTION

Histology slides sometimes referred to as microscope glass slides are objects usually prepared to demonstrate or explore the microscopic structure of cells, tissues and organs as well as to understand how the structure relates to function. These objects are often biological specimens secured or held on thin flat pieces of glass and most times stained to highlight various structural features, for visualization and evaluation with the aid of a microscope. A photomicrograph or simply micrograph (often generated from the glass slide produced) is a photograph taken through a microscope to show a magnified or digital image of a specimen or an object. Thus, it is a graphic reproduction of the image of an object formed by a microscope (Connett, 2017). The technique or process by which images of specimens are captured or photographed through a microscope is known as photomicrography.

The availability of a collection of histological glass slides and photomicrographs for review is essential in the study and practice of anatomy and other microscope-based disciplines including pathology and molecular biology. It is imperative for scholars to know how to prepare tissue samples as well as apply the knowledge to interpret cells and tissues through a microscope, rather than reproducing the information found in histology textbooks. However, due to the technical know-how required and/or the difficulty often faced in preparing or obtaining good quality slides as well as the high cost of purchasing ready-made slide collections for teaching and demonstration, histology is often taught today without laboratories, and a histology atlas is frequently used as a replacement. This is unfortunate because according to Sorenson and Brelje (2005), no matter how good the few images in a text book or histology atlas are, they cannot replace the experience of viewing a specimen through a microscope. This traditional method of histology which is centred on microscopy allows the observer an exceedingly close view of minute or very small structures at a scale convenient for examination and analysis (Ford and Shannon, 2021).

Over the years, major revisions within the medical teaching curricula of many institutions have placed severe constraints upon the time allocated to the teaching of traditional laboratory courses in the anatomical disciplines (Fitzharris, 1998; Hightower *et al*., 1999; Cotter, 2001). Despite the wealth of real and virtual imaging capabilities available to students in some institutions in developed countries, the most important resource to stimulating learning remains the one-on-one or small group interactions of students with faculty (Heidger, 2002). Hence, that approach helps to maintain the teaching of histology by using microscope glass slides and photomicrographs. However, a common problem facing many anatomists and other concerned laboratory scientists is the lack of a comprehensive practical guide to produce histology slides whenever needed. Moreover, the world of science is fast advancing beyond theoretical knowledge. Hence, this research project has been designed to rekindle scientific or practical interest in traditional methods of histopathology as well as to encourage efficiency or mastery of slides and photomicrographs production for the purpose of visualizing the real microstructure of cells, tissues and organs.

MATERIALS AND METHODS

Various materials in the form of reagents, instruments, tools and/or equipment were used and these included formalin for tissue fixation, alcohol or ethanol for dehydration, xylene for clearing tissue, paraffin wax for impregnation and embedding, DPX mountant for keeping specimen in place and protecting from accidental contact, glycerin for making section adhesive, dyes for staining, processing cassettes, embedding mould, water bath, frosted slides, staining racks and dishes, cover slips, rotary microtome, electric hot plate, hot air oven, plastic sample bottles, dissecting set, digital camera and light microscope.

The following step-by-step procedures constitute techniques for preparing histological tissue specimens as modified from the methods of Slaoui and Fiette (2011):

Tissue Sampling

This process involves the collection of tissue either by biopsy, surgical excision or postmortem dissection. Access to human tissue is not always easy or practical, and is complicated by ethics and legislation over both data and tissue samples (Thomas, 2014). For this research project, laboratory rats were euthanized through cervical dislocation to enable dissection and

extraction of various organs including different brain portions (cerebral cortex, cerebellum, hippocampus), heart, pancreas, liver, spleen, stomach, kidney, testis, seminal vesicle, prostate gland, ovary, breast and uterus. However, it is important to note that whatever method of euthanasia chosen in any case (i.e., depending on the research purpose) must follow scientifically accepted procedures.

Tissue Fixation

This procedure is necessary to preserve the tissue from decay or to keep it in a near life-like state as possible while also making it firm. Start fixation process immediately after the removal of selected organs to prevent decomposition caused by microbial activity.

I. Trim tissue/organs to reach the adequate size for rapid fixation
II. Label sample bottles or vials indicating sample name and collection date
III. Fill the bottles with fixative though not to the brim but ensure level up to two third of the vial
IV. Put the organs in separate vials with histological fixative such as 10% formalin (if tissue is very delicate, use Bouin's fluid). *See endnote 1* for information on how to prepare fixative.
V. Leave the tissue to fix depending on the appropriate duration or the type of fixative used and the temperature condition (formalin: 10-24 hours; Bouin's fluid: 4-8 hours, at room temperature)
VI. Next trim the tissue to obtain 3 – 5mm thick sample size that is compatible with subsequent histology steps as well as to get the proper orientation.
VII. Place the sample in a tissue processing cassette together with the appropriate label and then cover it properly. (When making labels during this step, use pieces of paper from plane sheet and make inscription with pencil. Do not use ink. Also, ensure the inscription on the label shifts toward the right half of the piece of paper leaving the left half space blank. This helps to retain the identity of the sample-specimen as it is passed through subsequent steps).

Dehydration

This procedure is carried out to remove water from the tissue and involves the immersion of samples in ascending grades of alcohol. S*ee end note 2 for guide to prepare alcohol solution*

I. Immerse in 50% alcohol for 45 minutes; afterwards use forceps to transfer sample to the next grade of alcohol.
II. 70% alcohol for 45 minutes
III. 90% alcohol for 45 minutes
IV. 100% (absolute) alcohol for 45 minutes
V. Absolute alcohol (second change) for 30 minutes
VI. Absolute alcohol (third change) for 30 minutes

Clearing

This step is necessary to remove alcohol from the tissue prior to paraffin embedding, using an agent that is miscible both in alcohol and paraffin. This treatment most often involves immersion of sample in xylene which also increases the refractive ability of the tissue by giving it a transparent appearance.

I. Immerse in xylene for 1 hour
II. Repeat the process in two changes of xylene for 30 minutes each.

Some other agents may be used for clearing but the time would vary as tissues would need to be kept for a longer period. These include:

a. Chloroform: widely used for its hardening effect; ideal for hard and delicate tissue
b. Toulene: tissues can be kept in this agent for a longer period as it causes less shrinkage
c. Cedar wood oil: preferred for delicate tissue due to its low penetrating rate.

Impregnation

This process is carried out to infiltrate the tissue sample with molten paraffin wax to replace clearing agent and provide support.

I. Use glass containers or aluminum jars to melt wax in hot air oven at a temperature of 60 – 67^{0}C. (Do not exceed this temperature to avoid tissue frying and shrinkage)
II. Transfer the tissue while enclosed in the processing cassette to molten wax for 20 minutes.
III. Repeat this process with another two changes in molten paraffin wax for 20 minutes each to completely remove the clearing agent.

Note: Ensure not to allow the wax to solidify when transferring the tissue from one change to the other; keep the wax liquefied by adding molten wax at regular intervals.

Embedding

This enables the careful positioning of the tissue inside a metal base mold or mould filled with melted paraffin.

I. Pour into the mold an adequate amount of molten wax heated at 65^{0}C.
II. Use warm forceps to place the tissue in the mold and adjust it appropriately to orient tissue surface toward the base of the mould.
III. Next, submerge the mould into cold water at 20^{0}C or place on a cooling surface such as the cold plate to avoid wax crystallization.
IV. Allow the blocks for a little time to harden and then remove them from the mould
V. Trim or cut the paraffin blocks into suitable sizes using a surgical blade or small knife.
VI. Next, melt the top surface of paraffin block with a heated knife and then mount it on a wooden block to firmly attach.

Sectioning

This is a process in which the tissue is cut from the paraffin block with a microtome. When ready to section a paraffin embedded organ or tissue:

I. Insert the appropriate knife in the holder or change disposable blade if necessary and tie or screw properly. Ensure to tie the knife clamp screw securely
II. Apply ice to the surface of the block for a few seconds and wipe off the water from the block surface. This is done to aid sections cut easily
III. Place or clamp the paraffin block in the microtome by pulling the medial arm forward, in a position away from the knife or blade to avoid accidental cut through the tissue.
IV. Adjust the microtome stage so as to move the paraffin quadrate close to the microtome plate
V. Unlock the microtome wheel with the appropriate button.
VI. Next place both hands on either side of the finger guard and pull it down as to reveal the microtome plate. (Always lock the wheel back into place when not sectioning).
VII. Use the section thickness adjustment knob to set initially to approximately 15µ (microns) so as to trim any excess wax and thereby expose a suitable area of tissue for sectioning.
VIII. After exposing a suitable area of tissue, set the section thickness to 5µ. (The appropriate thickness level for routine purposes is between 3 and 5 µ).
IX. Move the tissue surface of the block parallel to the edge of the knife to obtain straight ribbon of sections.
X. Turn the wheel to begin to cut into the paraffin specimens and continue turning until a desired section is obtained, making a ribbon that contains multiple sections of the tissue.
XI. Use twizers or small forceps to collect the paraffin ribbons from the microtome and then place on the slide that has been smeared with adhesive.
XII. Gently pour 30% alcohol upon one end of the slide and tilt it obliquely to enable the solution to spread over the ribbons and thus facilitate their flattening out during the subsequent step.

XIII. Next, place the ribbon to float on a water bath regulated at 45^0C so as to flatten or straighten the ribbon and also remove any shrinkage.
XIV. Use a blade or forceps to split the ribbon of sections floating on water to obtain individual or groups of sections as desired.
XV. Prior to picking up tissue sections that float on water to mount on a blank slide, first apply some adhesive on the frosted surface of the slide, as noted in XI above (*see end note 3 for a guide to prepare adhesive*).
XVI. When picking up the section from water, immerse the adhesive-smeared slide vertically to about three-fourths the length of the slide to bring the section in contact with it.
XVII. Lift the slide vertically from the water to find the section flattened on to the slide
XVIII. Blot sections lightly with blotting paper to remove excess water and to increase contact between slide and section, or keep the slide in upright position for several minutes to drain.

Staining

This involves a series of protocol aimed at colouring specimens to highlight important features of the tissue as well as to enhance the tissue contrast. The following steps are required for Hematoxylin and Eosin (H/E) method.

I. Prepare each dye or stain separately and put in a staining dish *(see end note 4a and b, for guide to prepare H/E stock solutions respectively).*
II. Arrange the glass slides in a staining rack.
III. Immerse the tissue sample in xylene for 10 minutes.
IV. Rinse the sample in fresh xylene for 5 seconds.
V. Rinse in absolute (100%) alcohol for 10 seconds.
VI. Rinse in another absolute alcohol for 5 seconds.
VII. Rinse in 90% alcohol for 5 seconds.
VIII. Rinse in 70% alcohol for 5 seconds.
IX. Rinse in 50% alcohol for 5 seconds
X. Rinse in tap water twice for 5 seconds each.
XI. Next, immerse sample in hematoxylin neutral solution for 15 minutes (*see end note 4a*).
XII. Dip in 1% acid alcohol to differentiate or get rid of excess stain *(see end note 5, on how to prepare the acid alcohol).*
XIII. Rinse in water twice for 5 seconds each.
XIV. Next, immerse in eosin for 5 minutes.
XV. Dip in water to rinse for 2 seconds.

Mounting and placing coverslips

This involves covering the tissue section in an ideal resin medium such as DPX or Canada balsam to keep specimen in place and protect it from accident contact.

I. Use a dispenser rod to apply 1 or 2 drops of DPX on the slide while being placed on a level surface.
II. Place the cover slip gently from one side of the droplet to avoid any air bubble inside it.
III. Put slides in a warmer, or just expose them to normal room air to dry for at least 6 hours and then cut excess medium from the edges of cover slip with a razor blade.

Photomicrography

This is a process by which images of specimens of interest are captured using a camera attached to a microscope that is connected to computer interface. Photomicrographs of histological tissue can be taken using Motic image plus 2.0 ML – the model 35 moticam camera

I. Fix the camera to the eye-piece of the photomicroscope to view the tissue through the various objectives of the photomicroscope.
II. Connect a lap-top as computer interface to the photomicroscope to aid viewing.
III. Observe the image of the tissue viewed on the lap-top screen.
IV. Capture the image and store for evaluation or studies as desired.
V. For each sample of tissue under study take photomicrograph at low power objective x10 and at high power objective x40 of the areas for study.

RESULTS

The following histology slides and photomicrographs are sample products of the research project:

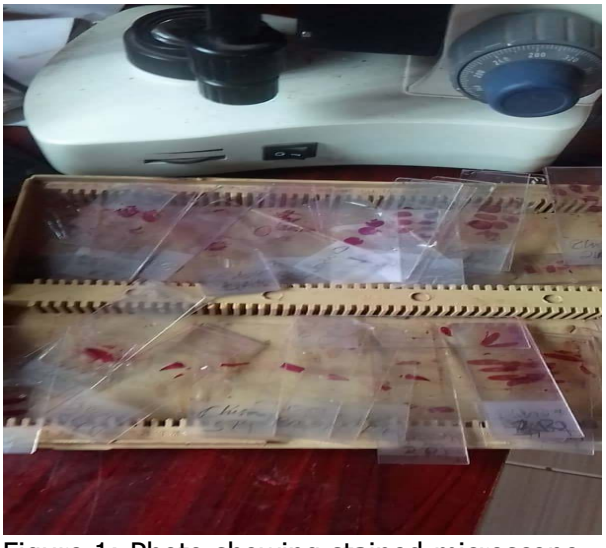

Figure 1: Photo showing stained microscope glass slides ready for photomicrography

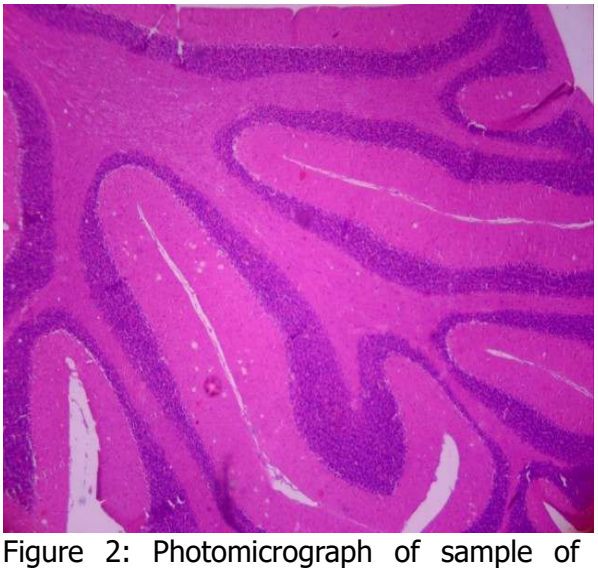

Figure 2: Photomicrograph of sample of cerebellum transverse section x400 (high magnification)

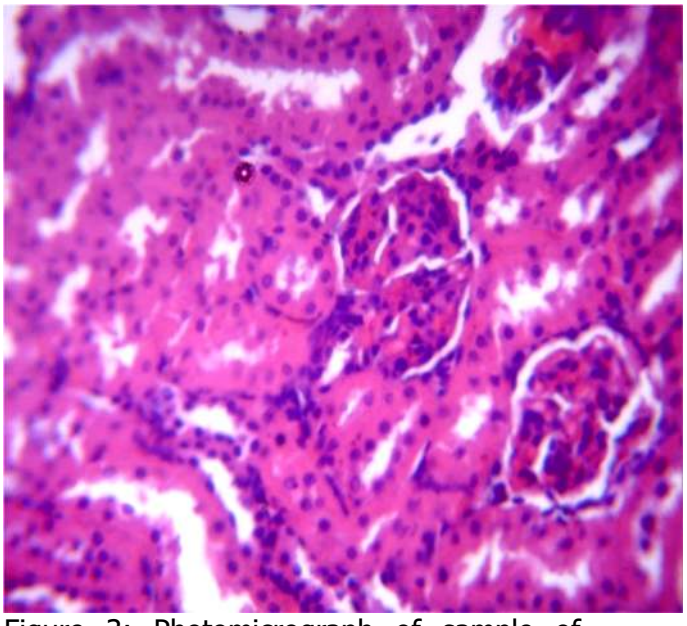

Figure 3: Photomicrograph of sample of kidney x400

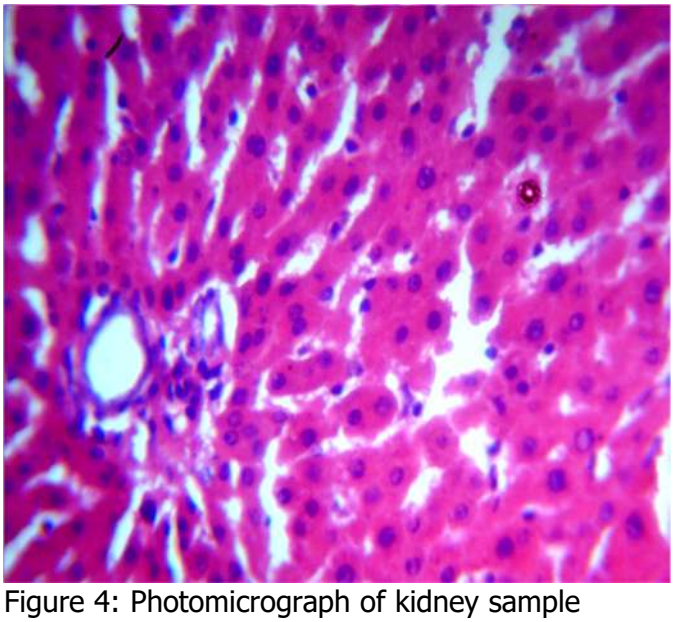

Figure 4: Photomicrograph of kidney sample x100 (low magnification)

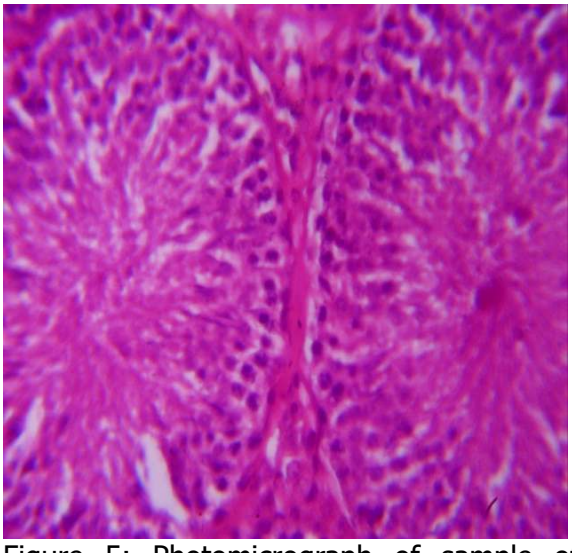

Figure 5: Photomicrograph of sample of testis (x400)

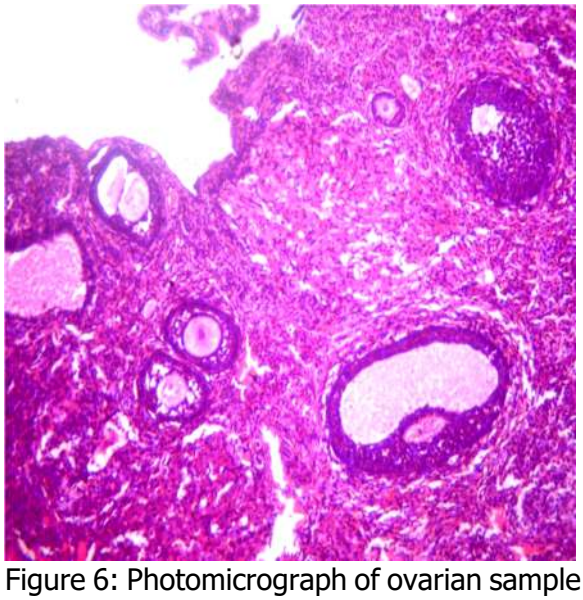

Figure 6: Photomicrograph of ovarian sample (x400)

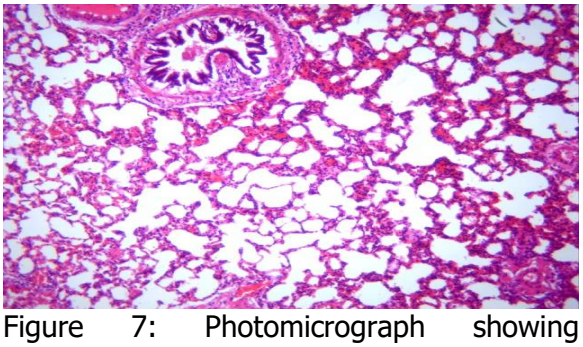

Figure 7: Photomicrograph showing longitudinal section of lung sample (x400)

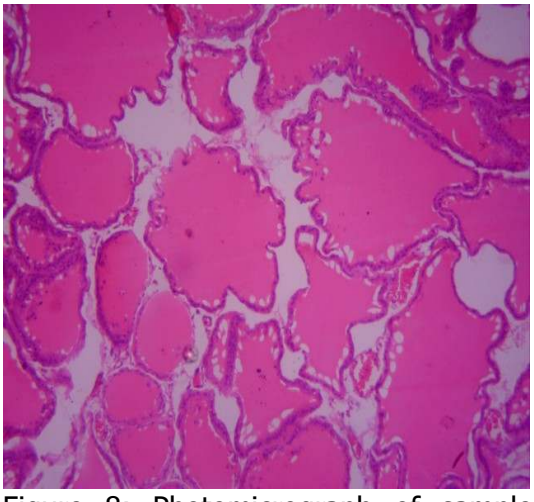

Figure 8: Photomicrograph of sample of prostate gland x400

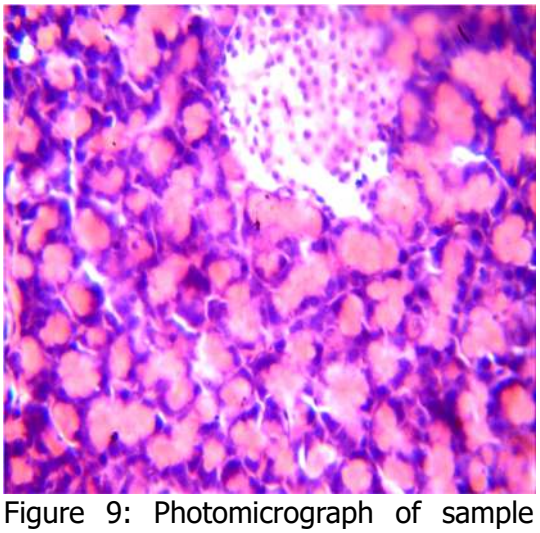

Figure 9: Photomicrograph of sample of pancreas (x100)

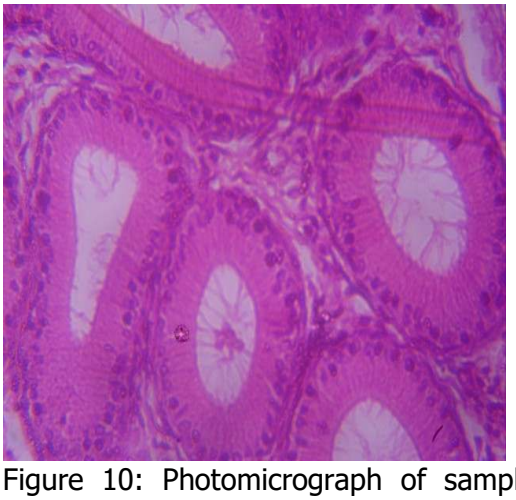

Figure 10: Photomicrograph of sample of epidydimis (x400)

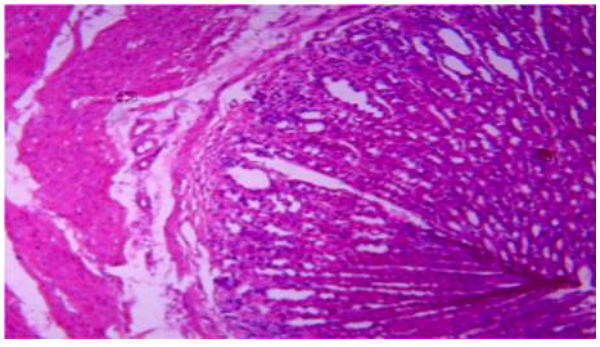

Figure 11: Photomicrograph showing longitudinal section of stomach sample (x100).

DISCUSSION

As presented in figure 1, glass microscope slides were produced by paraffin method of histological tissue preparation. The various tissue specimens or samples were secured or mounted on the slides. This arrangement enables a slide-mounted specimen to be quickly inserted in the microscope for examination and thereafter removed for storage in the appropriate slide box. The traditional glass slide with the aid of a microscope serves to give the observer an extremely close view of very small structures at a scale convenient for proper evaluation. The origin of the concept was pieces of ivory or bone, containing specimens held between disks of transparent mica that would slide into the gap between the stage and the objective of a microscope). These "sliders" were popular in Victorian England until the Royal Microscopical Society introduced the standardized glass microscope slide (Connett, 2017).

Apart from enabling close attention to structural details when viewed under the microscope, images of specimens are captured or photographed at either low or high magnifications as shown in figures 2-11. Despite the variation in format and content between institutions offering anatomical sciences and/or other microscope-based courses, light microscopy is still the most important tool for practical teaching and photomicrographs remain the mainstay of self-study material (Marshall *et al*, 2004). Even as digitized images and virtual slides become readily available, the stained glass slide has for centuries been the mainstay and its examination will remain the "gold standard" (Tsai *et al*., 2007). Since optical instruments were first used in the early 17th century to visualize biological material at the microscopic scale, the morphological study of cells and tissues has largely been driven by technological advances, such as the development of high quality light microscopes, new procedure for preparing biological specimens for visual analysis and novel microscope equipment and approaches (Hortsch, 2013).

For many years, the teaching of microanatomy has relied on banks of light microscopes and boxes of histological glass slides. This gave students an opportunity to learn how to operate a light microscope. However, this approach suffered from a great variability in the quality of the slide material, even as good quality histology glass slides are often difficult to obtain or prohibitively expensive. In addition, many tissue and organ preparations are difficult to come by. These problems and the upkeep of many light microscopes are a continuous expense, especially during times of diminishing financial support for higher education (Drake *et al*., 2009; Horsch, 2013). Even in settings where virtual microscopy tends to be replacing light microscopes and glass slides for the teaching of histology, there are limitations as access to a single virtual microscopy website relies on a fast,

stable internet connection and its contents may not align with different curricular and course structures (Logan *et al*, 2009).

Over the last ten years, many schools and universities in some countries have moved away from the use of real microscopes and have adapted a novel, electronic way to expose student to the structure of cells and tissues at the microscopic scale (Coleman, 2009; Drake *et al*., 2009). Although this emerging modality known as virtual microscopy is perceived as a useful tool for learning, the hybrid or traditional approach still remains the most preferred for histology learning (Telang *et al*, 2016). According to Heidger (2002) the contention is that, despite the wealth of real and virtual imaging capabilities available to students of histology, the most important resource to stimulating learning remains the one-on-one or small group interaction of students with faculty, as this approach helps to maintain the teaching of histology by traditional means.

There are many reasons to examine human cells and tissues under the microscope. Medical and biological research is underpinned by knowledge of the normal structure and function of cells and tissues, and the organs or structures that they makeup. In the normal healthy state (as visible in the photomicrographs in figures 2-11), the cells and other tissue elements are arranged in regular, recognizable patterns. However, in the event of changes induced by a wide range of chemical and physical influences, these are reflected by alterations in the structure at the microscopic level, and many diseases are characterized by typical structural and chemical abnormalities that differ from the normal state.

Without a sound knowledge of normal histomorphology as obtained in the present research, it is impossible to identify these changes and link them to particular diseases that should form the basis of important specializations of modern medicine such as histopathology and cytopathology. Microscopy plays an important part in haematology, microbiology and more broadly in the areas of biology, zoology, and botany because all these disciplines involve the examination of specimens under the microscope (Rolls, 2021). The availability of high quality digital consumer cameras at relatively low prices has made photography with the microscope significantly easier than with traditional film, together with recent developments in software aimed at amateur digital images, which now offers the photomicrographer capabilities for images that were not possible only a few years ago (Quekett, 2021).

CONCLUSION

The output of this research project is relevant in scientific education, due to the ease of reproducibility and replication of the techniques as well as the use of its products for teaching and practical demonstrations.

ENDNOTES

1. Preparation of 10% formalin: requires 1 part of stock formalin (i.e formaldehyde solution) with 9 parts water. For example when using a 100 mL calibrated cylinder, simply measure 10 mLs of formalin and then add 90 mLs of water.
2. Dehydrating stock solution constitutes ethanol and water. To get a particular concentration, apply the formula $V_1=C_2V_2\div C_1$ where V_1 and V_2 represent initial and final volume respectively while C_1 and C_2 stand for the initial and final concentration. Example, to prepare 60% ethanol in a final volume of 100 mL: V_1=?, V_2=100mL, C_1=100%, C_2 =60% $V_{1=}C_2V_2\div C_1$.

Therefore (60%)(100mL)÷100% =60 mL. A quick way is to divide 60 by 100% and multiply by 100mL (which is the total volume of container). This yields a 60mL measure of ethanol, while the remaining 40 mLs is of water.

3. Section adhesive preparation: mix 50% glycerin with 50% albumin (fresh egg white) to make this.

4. Stains

4a. Hematoxylin preparation: Either of two methods can be used. One of these is the progressive method which requires the addition of excess acid or salts in the solution to increase the accuracy of nuclear staining. The other method known as regressive staining requires a neutral hematoxylin solution to over stain the tissues; afterwards wash the slides by dipping in acid to remove excess stain and then rinse in water to neutralize the acid wash.

I. Dissolve 5 g of hematoxylin in 50 ml of absolute alcohol.
II. Dissolve 100 g of potassium alum in 1000 ml of distilled water by heating.
III. Mix the two solutions obtained in the preceding steps i and ii and then heat as fast as possible so as to boil within one minute while stirring at frequent intervals.
IV. Next, remove from heat and add 2.5 g of mercuric oxide slowly.
V. Heat again to boil slowly until it turns dark purple,
VI. Remove from heat and immediately thrust the kettle or flask into cold water and allow to cool. Afterwards the stain can readily be used.

However, if the progressive method is chosen, about 4 ml of glacial acid per 100 ml of solution should be added and filtered before use.

4b. Eosin preparation: i. dissolve 1 g of the dye in 80 ml of distilled water. ii. mix the solution with 320 ml of 95% alcohol. iii. Add 0.4 ml or a few drops of glacial acetic acid.

5. Preparation of 1% acid alcohol when using a total volume of 100 mL, requires the addition of 99 mL of 70% ethanol to 1mL of hydrochloric acid (HCL).

ACKNOWLEDGEMENT

The Authors acknowledge funding support from Tertiary Education Trust Fund (TETFund) of Nigeria, by means of institution-based research grant, with reference number: TETF/DR&D/CE/UNI/CAL/RG/2021/VOL.1

CONFLICTS OF INTEREST

The authors affirmed that there are not any conflicts of interests

REFERENCES

1. Coleman R. (2009). Can histology and pathology be taught without microscopes? The advantages and disadvantages of virtual histology. *Acta Histochem*; 111:1-4. DOI: 01:10.1016/j.acthis.200809.003.
2. Connett J. (2017). The art of the invisible. *Bristol 24-7* Available: www.bristol247.com//lifestyle/shops/the – art of –the invisible/[Accessed 2021 May 15].
3. Cotter JR. (2001). Laboratory instruction in histology at the university at Buffalo: Recent replacement of microscope exercises with computer applications. *Anat Rec*; 265**:**212-221.
4. Drake RL, McBride JM, Lachman N, Pawlina W. (2009). Medical education in the anatomical sciences. The winds of change continue to blow. *Anat Sci Educ;* 253-259. DOI: 10./1002/ase.117.
5. Fitzharis TP. (1998). Survey of gross anatomy courses in the United States and Canada. *Anat Rec (New Anat)*; 253:163-166.
6. Ford BJ, Shannon RR. (2021). Microscope instrument. Available:

https://www.britanica.com/technology/microscope [Accessed 2021 May 11].
7. Heidger PM, Dee F, Consoer D, Leaven T, Duncan J, Kreiter C. (2002). Intergrated approach to teaching and testing in histology with real and virtual imaging. *Anat Rec*; 269:107-112.
8. Hightower JA, Boockfor FR, Blake CA, Millett CF. (1999). The standard medical microscopic anatomy courses: Histology circa 1998. *Anat Rec (New Anat)*; 257:96-101.
9. Hortsch M. (2013). Virtual biology: teaching histology in the age of Facebook. *Fed Am Soc Exp Biol FASEB J*; 27 (2): 411-413.
10. Logan R, Baron J, Swann C. (2009). Oral pathology in blended space: A pilot study in same places, different spaces. Available: http//www.asclite. org.au/conferences/auckland09/procs/logan-poster.pdf [Accessed 2021 May 14].
11. Marshall R, Cartwright N, Mattick K. (2004). Teaching and learning pathology: A critical review of English literature. *Med Edu*; 38:302-13.
12. Quekett Microscopical Club (2021). Advanced digital photomicrography. Available: https://www.quekett.org/resources/advanced-photomicrography [Accessed 2021 May 16].
13. Rolls G. (2021). An introduction to specimen preparation. Available: https://www.leicabiosystems.com/knowledge-pathoway/an-introduction-to-specimen-preparation/ [Accessed 2021 May 15].
14. Slaoui M, Fiette L. (2011). Histopathology procedures: from tissue sampling to histopathological evaluation. *Methods Mol Biol*; 601:69-82. DOI:10.1007/978-1-60761- 849-2_4
15. Sorenson RL, Brelje TC. (2005). Atlas of human histology: A guide to microscopic structure of cells, tissues and organs. Available: www.histologyguide.com [Accessed 2021 May 12]
16. Telang A, Jong ND, Dalen JV. (2016). Media matter; The effect of medium of presentation on student's recognition of histopathology. *J Clin Diagn Res*; 10 (12): 1-5. DOI: 10.7860/JCDR/2016/22208.8969
17. Thomas G. (2014). Access to human cells and tissues: Chapter 1 in Human-based Systems for Translational Research; Pp 1-16**.** DOI: 10.1039/9781782620136-00001.
18. Tsai S, Kartono F, Shitabata PK. (2007). A novel glass slide filling system for pathology slides. *Am J Clin Pathol*; 128:109-111. DOI: 10.1309/104F7E17XmFEOTXX