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Original Article

Design and Fabrication of Portable Automatic Tissue Processing Machine

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Abstract *- Tissue-sectioning automation can be a resourceful tool in processing anatomical pathology specimens. Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures. In most developing countries, including Nigeria, however, access to this automated equipment is encumbered by a lean healthcare budget. The aim of this study, therefore, is to design and fabricate a portable and cheap automated tissue processing machine with locally sourced materials. The programming sequence used in this study is; Flow chart design., Developing the microcontroller's source code using micro basic language., Debugging the micro basic code, Compiling the source code with a compiler and creating a hex file (i.e. the file extensive is "hex") and Loading the hex file of the programme (Top universal programmer [Top2008]) into the microcontroller's (atmega32) memory. We fabricated an automated tissue processing machine to process tissues between 2-3 mm thick. Comparing slices made from our design and that from a Histokinette ATP300 closure: a conventional tissue processor, we conclude that our design is efficient.*

Keywords *- Automated, Fabrication, Microcontroller, Processing, Sectioning, Tissue.*

1. Introduction

Tissue-sectioning automation can be a resourceful tool in processing anatomical pathology specimens. The advantages of an automated system compared with traditional manual sectioning include invariable thickness, uniform orientation and fewer tissue-sectioning artifacts (Onozato, Hammond, Merren, & Yagi, 2013, Jaeger et al., 2016). Microscopic examination of cellular morphology (Okafor et al., 2021) and structure of tissues is a classical approach that has provided an invaluable foundation for analyzing the function, development, and organization of complex tissues (Ferté, André, & Soria, 2010, Galli et al., 2014). Accordingly, a large number of biomedical research and diagnostic methods are based on the identification of architectural tissue features by histopathology (Lassmann & Werner, 2012, Matthäus et al., 2010, Okafor et al., 2022b).

At the same time, highly multiplexed interrogation of the molecular components of different samples has proven to be a tremendously rich complementary strategy for their characterization and classification (Alizadeh et al., 2000). Large-scale molecular studies based on microarray analysis, high-throughput sequencing, and proteomic approaches have clearly demonstrated the advantages of quantitative multi-dimensional profiling for identifying functionally important subtypes of cancers and other cellular states with important clinical ramifications (Matsuda et al., 2010, Bendall et al., 2011). It is, therefore, impossible to underplay the importance of fixation in histopathology. Whether the scientist is interested in extracting information on lipids, proteins, RNA or DNA, fixation is critical to this extraction. The veritable tool for this procedure is the automatic tissue processor (Howat & Wilson, 2014, Potora et al., 2014)).

A tissue processor is a device that prepares tissue samples for sectioning and microscopic examination (Fukumoto & Fujimoto, 2002, Mariani et al., 2009). Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures; hence the tissue processor plays a big role in the preparation of tissue by passing them through various chemicals (Titford, 2006, Steuwe et al., 2014)). Although this device is slowly evolving, its cost, size, weight, maintenance, and mobility have necessitated the fabrication of a cost-effective, user and space-friendly tissue processor.

2. Materials and Method

2.1. Materials

Table 1. List of electronic components

S/NO	Components Description	Quantity	Specification
1	Microcontroller	1	Atmega32, 20mA per bit terminal, 5V, 40pin terminals.
2	IC socket	1	40 pin
3	Resistor	3	1K and 10k, 0.5W
4	Capacitor	3	22pF/35V and 1000uF
5	DC motor	2	12V
6	Transistor	3	BC547
7	L C D	1	LM3229, 5V
8	Veroboard	1	12 *30 holes
9	Lead	10Yards	
10	Jumper Wire	2 Yards	RJ45
11	Transformer	1	220V-12V
12	Optical sensor	12	3-5V
13	Variable resistor	2	10k, 0.5W
14	SPST switch	2	10A, 220V
15	Regulator	1	7805 type, 5V,1A
16	Diode	5	1N4007,7A
17	(LED)	1	5mm, 1.5V-3V, 20mA
18	Switch	2	Push to make (Micro)
19	Crystal oscillator	1	20MHz
20	relay	2	12V, 30A

2.2. Method

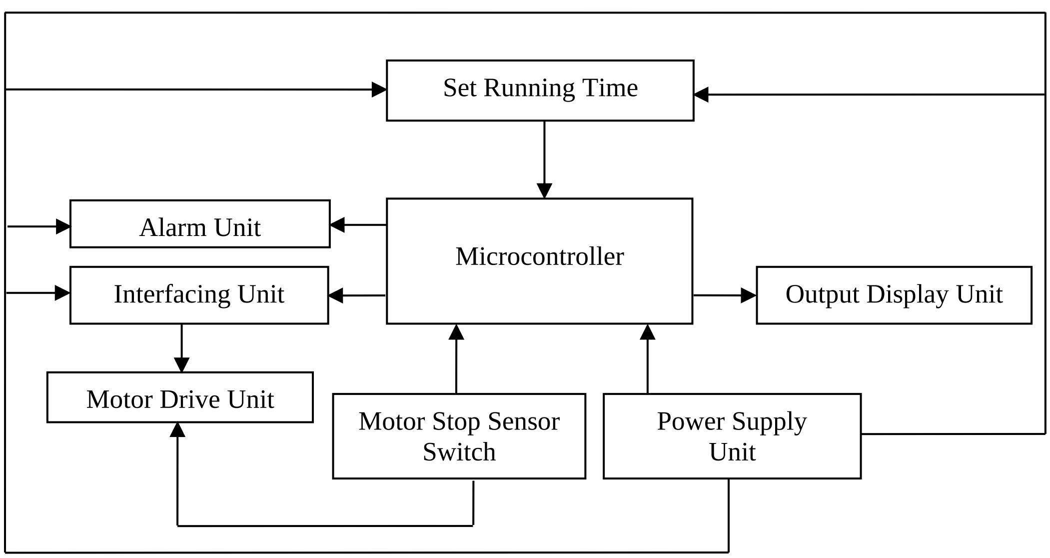

Fig. 1 Block diagram of tissue machine

2.2.1. Set Running Time

In this section, the method used by Okafor *et al*., 2022 and Sixtus *et al*., 2022 is modified by ensuring that the micro-controller stores the time interval the device is meant to run with. Microswitches were used to achieve that. The microcontroller is left to take decisions based on the stored information. A pull-up resistor is attached to the pin of the microcontroller to vcc, which helps to improve the current capacity of the microcontroller switching pins for proper functionality.

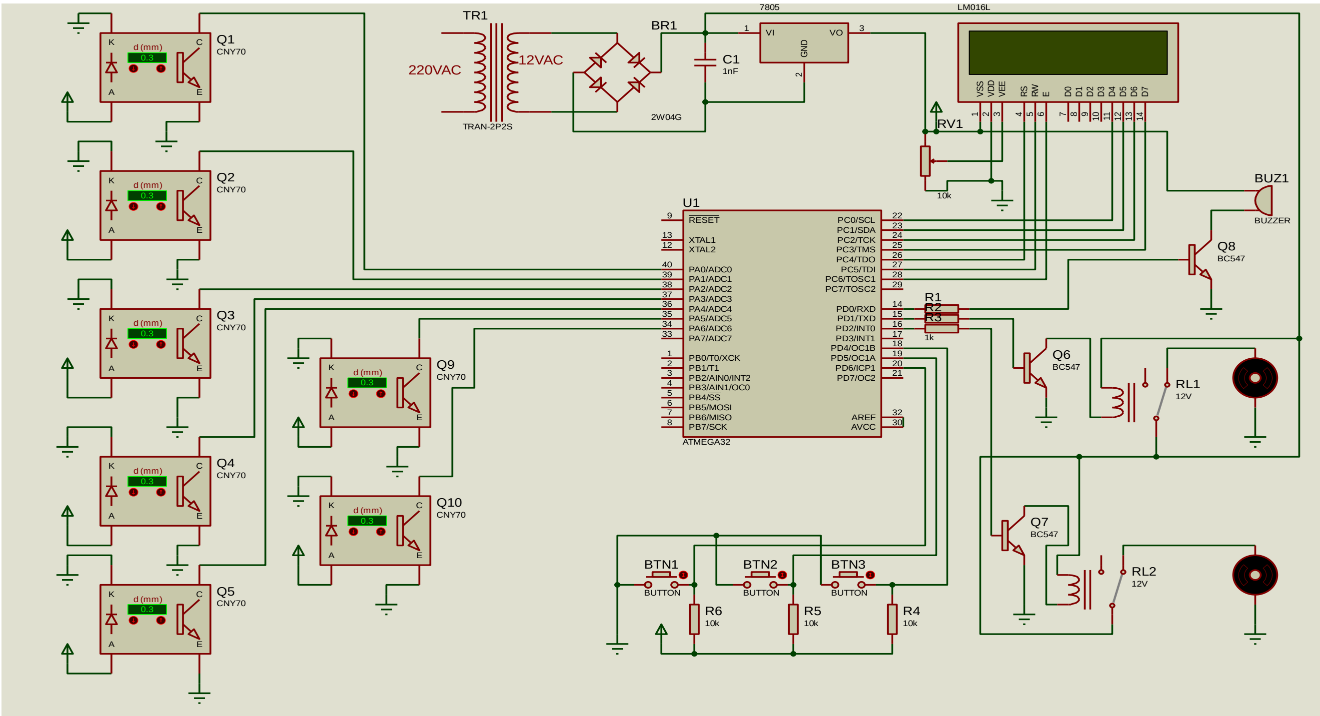

Fig. 2 Complete circuit diagram of automatic tissue processor

2.2.2. Alarm Unit

The alarm section triggers for 5 seconds once a complete circle is achieved. This unit ensures that the required current needed to control the alarm circuit is obtained. This interface serves as a link between the microcontroller and the alarm circuit. This is achieved with the help of the transistor configured in amplification mode.

2.2.3. Output Display Unit

The tissue processor uses a simple liquid-crystal display (LCD) driven by the microcontroller to display the system state at all times. The type of LCD used in this system is LM016L which runs on a power supply of 5V. The reason for using this LCD is to ensure that all data and information are captured and to enable easy communication between the device and the user.

2.2.4. Microcontroller Unit

This unit monitors and controls the behavior of the entire system. Each section of the entire system, both the input and output channel, has been monitored and controlled by the microcontroller unit as described by Okafor *et al.*, 2022. It accepts and interprets the accepted signals and takes its decision through the display unit. Micro-controller (Atmega32) maximum consumable current is 200mA (0.2A)

2.3. Fabrication

2.3.1. Electrical Stage

The microcontroller is the heart of the entire system; it interconnects all the different systems channels to ensure a working circuit. The system's input channel, which monitors the motor drive's dimension, is connected to port-A of the microcontroller. Port-C of the microcontroller is connected to the LCD data and control line, respectively. Three command buttons are connected from port-D.4 to port-D.6 of the microcontroller. Each pin serves as an input command pin that enables a function in the system duration operation (setting of time). A buzzer is connected to port-D.0 through a biasing Resistor to ensure that an output sound is generated.

Two relays that control the ON and OFF of the DC motor were interfaced with the transistor through a biasing resistor and connected to the microcontroller port-D.1 and port-D.2 respectively. 20MHz crystal oscillator is terminated at pins 12 and 13 of the microcontroller to establish a clock frequency the microcontroller needs for effective operation. Pin 9 of the microcontroller is connected to Vcc to reset the microcontroller at every complete cycle. Pin 30 and 32 are both connected to Vcc to activate the ADC channel of the microcontroller. The system comprises two voltages, 12V and 5V. The 7805 regulators are used to reduce the power supply voltage to a desired 5V output needed by the microcontroller and some other discrete components. A capacitor is used to filter the final output of the system.

Calculations

Using $I_B = V_{CC} – V_{BE} \div R_B$

V_{CC} = 5volts, the microcontroller voltage

R_B = 1000 ohms, the base resistor

Therefore

$I_B = V_{CC} – V_{BE} \div R_B$

$I_B = 5 – 0.7 ÷ 1000$

$I_B = 4.3 ÷ 1000$

$I_B = 4.3mA$

The transformer used is 220/24V, 50Hz, 1000mA

$V_{peak} = 24V$

$V_D = V_{peak} – (0.7 \times 2)$

$= 24 – (0.7 \times 2)$

$V_D = 22.6V$

$V_{CC} = 5volts$
$R_B = 330\ ohms$
Please note that

$V_B = V_{BE} = 4.2v$

Therefore

$I_B = V_{CC} – V_{BE} ÷ R_B$
$I_B = 5 – 4.2 ÷ 330 = 0.002424A$

The resistance of the relay =750 ohms
Voltage =12V
The current (I) needed by the relay coil for proper switching will be = 16mA.

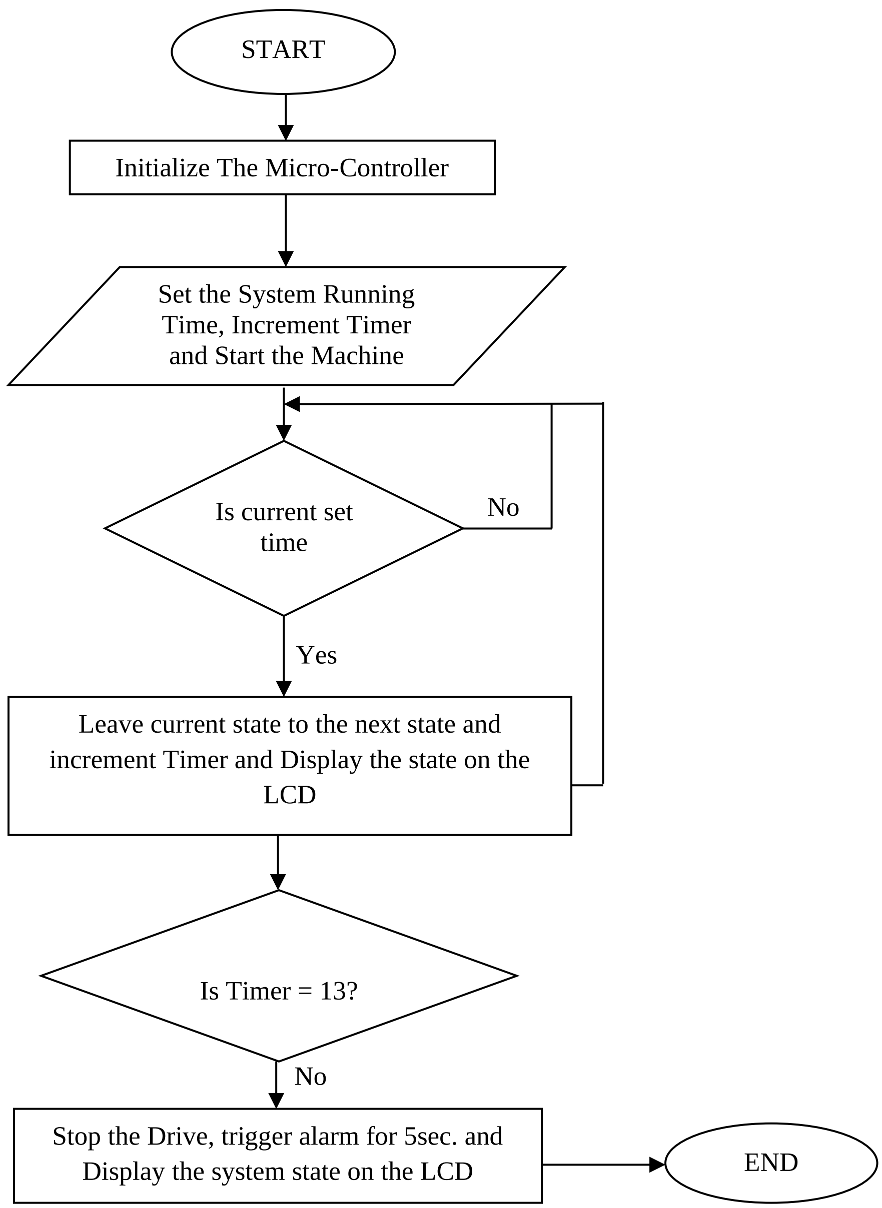

Fig. 3 System flow chart

2.3.2. Coupling Stage

The coupling stage has to do with the mechanical fabrication described by Sixtus *et al.*, 2022. First, the two motors used in this system are expected to drive in two different forms; the first motor drives in up and down motion serving as a lifting and closing drive for the tissue processor sample cover, while the second motor rotates the Tissue sample 360^0 with time and according to choice partition. To achieve the up-and-down drive, a Rack and Pinion were used to drive the tissue sample, while the second motor was attached to the upper body of the Rack. The two motor connections were enclosed with the body of the beaker stand to serve as a base for the beakers. The beakers were enclosed with a cover to avoid exposing the sample to atmospheric change.

2.4. Material Test

Table 2. Result of transistor test

S/NO	TEST CONDUCTED	RESULT (RESISTANCE)
1	Base to collect test	657
2	Base to emitter test	669

Table 3. Result of resistor test

S/NO	TEST CONDUCTED	RESULT (RESISTANCE)
1	For a 1K resistor	0.9974
2	For a 10K resistor	10.043
3	For 330 resistors	331

Table 4. Result for interfacing test

Test	Good State (V) Result	Bad State (V) Result
Connect 5V DC at the base terminal of the transistor through a biasing resistor.	The recorded output is 5V.	-
Connect 0V DC at the base terminal of the transistor through the biasing resistor to know if the transistor switches with both signals (0 and 1), which is improper.	The recorded output is 0V.	-

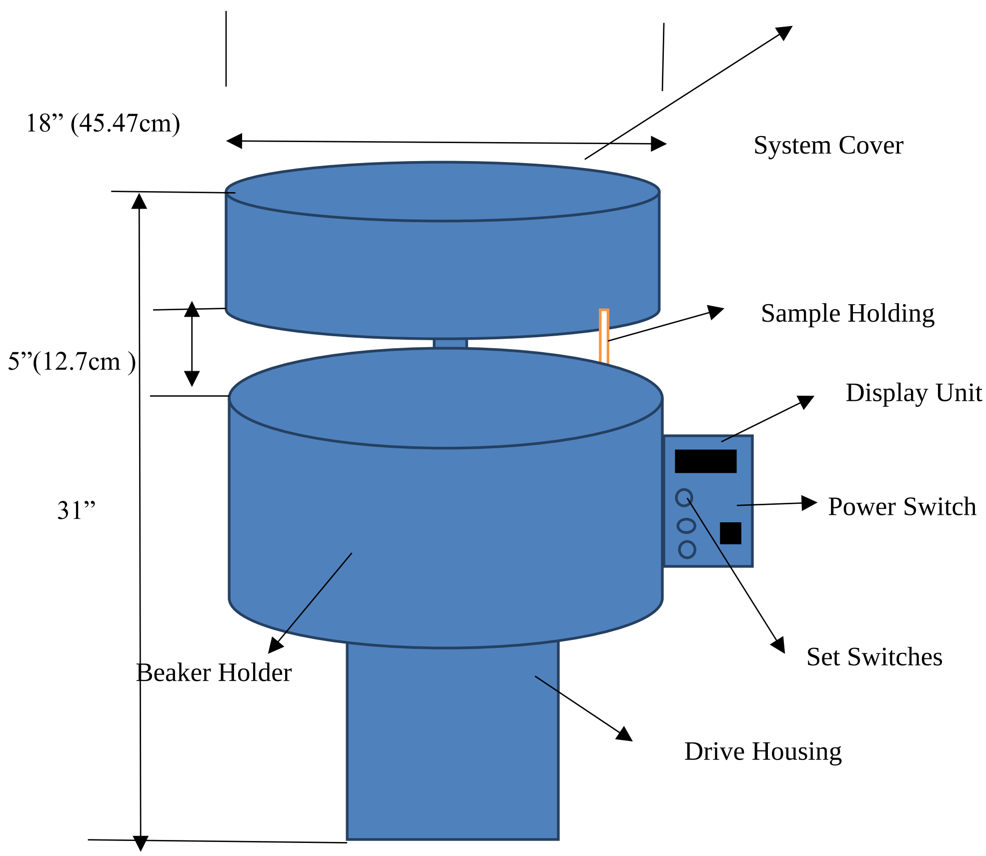

Fig. 4 Design specification

Table 5. System test and result

S/NO	TEST	RESULT
1	Press the power button	The device switches ON the screen and requests for the time interval setting.
2	After setting the time, press the OK button.	The device lifts up and down the tissue cover and rotates the motor at an angle of $360^0/12$. Then displays the current state of the system on the LCD. The process continues until a complete 360^0 rotations are achieved. At the end of the process, an alarm triggers for 5 seconds.
3	While the system operation is ongoing, Press any of the buttons except the power switch	The system remains in its current state, ignoring any incoming signal until the processing is completed, with tissue slices between 3 – 4 mm thick.

3. Result

3.1. System Test and Result

To acquire results, two different tests were conducted; the device test and the usage test.

3.2. Device Test

The device test involves running the system to ensure its readiness to function. Table 5 illustrates the test and results received during this process.

4. Discussion

In all Tissue Processing Machine that uses a microcontroller, various microcontroller programmable interfaces are available; however, the Top universal programmer (Top2008) has been in use. It is a universal programmer that can programme different categories of micro-controllers (Kuzmin *et al*., 2014, Rolls, 2019). However, our design used atmega32 memory, as described by (Okafor *et al*., 22a), which accepts and interprets the input signals and takes its decision through the display unit with the maximum consumable current of 200mA (0.2A).

The programmer was used to load the Hex file of the program into the memory of the microcontroller (atmega32) following these programming sequences: Flow chart design; Developing the microcontroller's source code using micro basic language; Debugging the micro basic code; Compiling the source code with a compiler and creating a hex file (i.e. the file extensive is "hex") and loading the hex file of the program into the microcontroller's (atmega32) memory.

The method used by Okafor *et al*., 2022 and Sixtus *et al*., 2022 is modified by ensuring that the micro-controller stores the time interval the device is meant to run with; micro switches were used to achieve that. The microcontroller is left to take decisions based on the stored information. A pull-up resistor is attached to the pin of the microcontroller to vcc, which helps to improve the current capacity of the microcontroller switching pins for proper functionality. Each pin serves as an input command pin that enables a function in the system duration operation.

The programme code was loaded into the micro-controller through the following steps: open the TOP2008 software; Go to load the file and locate the converted hex file; Choose the atmega32 microcontroller as the choice of the microcontroller to be used; Set the microcontroller clock frequency to 8MHz; Enable all the execution buttons (erase, write, verify, etc.) and Click on programme button. The LM016L Liquid–Crystal Display (LCD) which runs on a 5V power supply, was used to interface between our device and the user.

Our design is fitted with an alarm system that triggers for 5 seconds once a cycle is completed, achieved with the help of the transistor configured in amplification mode. Two relays that control the ON and OFF of the DC motor were interfaced with the transistor through a biasing resistor and connected to the microcontroller port-D.1 and port-D.2 respectively. As described by Sixtus et al., 2022, mechanical fabrication involves using two motors.

The first motor drives in up and down motion serving as a lifting and closing drive for the tissue processor sample cover, while the second motor rotates the Tissue sample 360^0 according to the choice partition. The two motor connections were enclosed with the body of the beaker stand to serve as a base for the beakers. The beakers were enclosed with a cover to avoid exposing the sample to atmospheric change. Upon fabrication and coupling, our design was used to process tissue and fine sections of tissue with thicknesses ranging between 3 to 4 mm was processed.

5. Conclusion

Although, significant variables in tissue processing include: the operating conditions, particularly temperature, the characteristics and concentrations of the reagents and the properties of the tissue. Our fabricated tissue processor has shown to be efficient compared with a conventional tissue processor, Histokinette ATP300 closure, as it was able to process very fine sections of tissue of thickness between 3 to 4mm.

References

[1] Maristela L Onozato et al., "Evaluation of a Completely Automated Tissue-Sectioning Machine for Paraffin Blocks," *Journal of Clinical Pathology*, vol. 66, no. 2, pp. 151-154, 2013. [CrossRef] [Google Scholar] [Publisher Link]

[2] Daniel Jaeger et al., "Label-Free in Vivo Analysis of Intracellular Lipid Droplets in the Oleaginous Microalga Monoraphidium Neglectum by Coherent Raman Scattering Microscopy," *Scientific Reports*, vol. 6, 2016. [CrossRef] [Google Scholar] [Publisher Link]

[3] S. A. Okafor et al., "Miscellany of Hospital Contact Surfaces Microbiome: A Case Study of Selected in Owerri South Eastern Nigeria," *African Journal of Medical Physics, Biomedical Engineering*, vol. 8, no. 2, pp. 48-57, 2021. [Google Scholar] [Publisher Link]

[4] Charles Ferté, Fabrice André, and Jean-Charles Soria, "Molecular Circuits of Solid Tumors: Prognostic and Predictive Tools for Bedside Use," *Nature Reviews Clinical Oncology*, vol. 7, pp. 367-380, 2010. [CrossRef] [Google Scholar] [Publisher Link]

[5] Roberta Galli et al., "Effects of Tissue Fixation on Coherent Anti-Stokes Raman Scattering Images of Brain," *Journal of Biomedical Optics*, vol. 19, no. 7, 2013. [CrossRef] [Google Scholar] [Publisher Link]

[6] Silke Lassmann, and Martin Werner, "Predictive Pathology in Routine Diagnostics of Solid Tumors," *Histology and Histopathology*, vol. 27, pp. 289–296, 2012. [CrossRef] [Google Scholar] [Publisher Link]

[7] Christian Matthäus et al., "Monitoring Intra-Cellular Lipid Metabolism in Macrophages by Raman- and CARS-Microscopy," *Proceedings of SPIE Photonics Europe*, vol. 7715, 2010. [CrossRef] [Google Scholar] [Publisher Link]

[8] Sixtus A. Okafor et al., "Investigating the Bioburden of "Neglected" Hospital Low Contact Surfaces," *Advances in Microbiology*, vol. 12, no. 5, 2022. [CrossRef] [Google Scholar] [Publisher Link]

[9] Ash A. Alizadeh et al., "Distinct Types of Diffuse Large B-Cell Lymphoma Dentified by Gene Expression Profiling," *Nature*, vol. 403, pp. 503–511, 2000. [CrossRef] [Google Scholar] [Publisher Link]

[10] Yoko Matsuda et al., "Comparison of Fixation Methods for Preservation of Morphology, RNAs, and Proteins from Paraffin-Embedded Human Cancer Cell-Implanted Mouse Models," *Journal of Histochemistry & Cytochemistry*, vol. 59, no. 1, pp. 68-75, 2011. [CrossRef] [Google Scholar] [Publisher Link]

[11] Sean C. Bendall et al., "Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum," *Science*, vol. 332, no. 6030, pp. 687-696, 2011. [CrossRef] [Google Scholar] [Publisher Link]

[12] William J. Howat, and Beverley A. Wilson, "Tissue Fixation and the Effect of Molecular Fixatives on Downstream Staining Procedures," *Methods*, vol. 70, no. 1, pp. 12–19, 2014. [CrossRef] [Google Scholar] [Publisher Link]

[13] Mariana C. Potcoava et al., "Raman and Coherent Anti-Stokes Raman Scattering Microscopy Studies of Changes in Lipid Content and Composition in Hormone-Treated Breast And Prostate Cancer Cells," *Journal of Biomedical Optics*, vol. 19, no. 11, 2014. [CrossRef] [Google Scholar] [Publisher Link]

[14] Satoshi Fukumoto, and Toyoshi Fujimoto, "Deformation of Lipid Droplets in Fixed Samples," *Histochemistry and Cell Biology*, vol. 118, pp. 423-428, 2002. [CrossRef] [Google Scholar] [Publisher Link]

[15] Melissa M. Mariani et al., "Impact of Fixation on in Vitro Cell Culture Lines Monitored with Raman Spectroscopy," *Analyst*, vol. 134, pp. 1154-1161, 2009. [CrossRef] [Google Scholar] [Publisher Link]

[16] Michael Titford, "A Short History of Histopathology Technique," *Journal of Histotechnology*, vol. 29, no. 2, pp. 99–110, 2006. [CrossRef] [Google Scholar] [Publisher Link]

[17] Christian Steuwe et al., "CARS based Label-Free Assay for Assessment of Drugs by Monitoring Lipid Droplets in Tumour Cells," *Journal of Biophotonics*, vol. 7, no. 11-12, pp. 906-913, 2014. [CrossRef] [Google Scholar] [Publisher Link]

[18] Sixtus Amarachukwu Okafor et al., "Fabrication of a Locally Designed Dual Power Supply Hand – Eye Coordination Tester with a Micro Controller IC Interface," *IOSR Journal of Engineering*, vol. 12, no. 5, pp. 1-9, 2022. [Publisher Link]

[19] Sixtus Amarachukwu Okafor et al., "Design and Development of an Adjustable Dynamic Hand Splint," *Engineering and Technology Journal*, vol. 7, no. 9, pp. 1449-1458, 2022. [CrossRef] [Publisher Link]

[20] Kuzmin, A. Pliss, P. N. Prasad, "Changes in Biomolecular Profile in a Single Nucleolus during Cell Fixation," *Analytical Chemistry*, vol. 86, no. 21, pp. 10909-10916, 2014. [CrossRef] [Google Scholar] [Publisher Link]

[21] Geoffrey Rolls, 2019. [Online]. Available: https://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-processing/