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Using Medical Imaging to Differentiate Live and Dead Sperm Cells: A Technical and Analytical Study

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Abstract: The purpose of the present study is to create an automated program of live and dead sperm cells sorting and classification through vital staining by using microscopic analysis and computer programs. The eosin-Nigrosin staining was used to assess the sperm viability, and the high-resolution image was acquired and analyzed through the use of image processing. This study offers a practical and accurate alternative to traditional manual evaluation by extracting visual data related to shape, color, and interaction with the dye. Based on the results, the proposed system can accurately distinguish between live and non-live sperm, improve the quality of samples used in assisted reproductive technologies, and reduce human intervention. This article represents a positive step towards the adoption of artificial intelligence in fertility laboratories to facilitate rapid, evidence-based decision-making.

Introduction

Accurate assessment of sperm motility has long been considered a cornerstone of reproductive medicine. Historically, the earliest attempts to treat male infertility date back to the late 18th century, when John Hunter in London performed one of the first documented cases of assisted reproductive technology (ART), inseminating a woman with a sample of her husband's semen. These pioneering efforts laid the foundation for (ART), which has since evolved into sophisticated procedures such as intrauterine insemination (IUI) and in vitro fertilization (IVF).[1] The scientific journey to assess sperm motility began with simple microscopic observations, where researchers relied on motility as an indicator of viability. However, motility alone was insufficient, as non-motile sperm could retain their fertilizing capacity. By the mid-20th century, bio-staining techniques were developed, using dyes such as eosin-nigrosin to distinguish between live and dead sperm based on membrane integrity. These methods provided a more reliable measure of sperm quality and became widely used in clinical practice.[2] The birth of Louise Brown in 1978, the first human born through in vitro fertilization (IVF), marked a turning point in reproductive medicine. Since then, the demand for accurate sperm evaluation methods has increased significantly, driven by the need to improve the success rates of assisted reproductive technologies. Over the past four decades, technological advancements have revolutionized sperm viability assessment, from manual staining and self-monitoring to automated imaging and computer analysis.[3] In recent decades, there has been a growing emphasis on the need for more accurate and precise sperm viability assessment to improve the success rates of assisted reproductive

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technologies, including intrauterine insemination (IUI) and in vitro fertilization (IVF). The ability to distinguish between live and dead sperm is a fundamental aspect of sperm quality assessment and has a direct impact on fertilization potential and treatment options. [4, 5] Determining sperm viability is a key aspect of reproductive medicine, particularly in fertility diagnosis and assisted reproductive procedures. Bio staining is a commonly used method for separating live sperm from dead sperm, where certain dyes are used to react with cell membranes depending on their integrity, making their visual separation possible through a microscope. [6,7]. After the staining procedure, an image of the medical system becomes very important in recording detailed visual information on the samples. The difficulty then changes over to the correct sorting and categorization of stained sperm cells by means of computer analysis. This study aims at creating and analyzing image-based methods to detect and separate dead and alive sperm following the use of staining methods using the traditional image processing as well as the contemporary machine learning algorithms. [8,9,10,11]. Through a staining analysis of spermatozoa, the research will enhance the objectivity and automation of sperm viability classification. It aims at decreasing human bias, enhancing the accuracy of sorting and providing scalable solutions to clinical implementation through feature extraction, segmentation, and classification models [12]. The integration of imaging and computational methods holds promise for enhancing the efficiency and reliability of sperm selection in fertility laboratories, the research aims to contribute to the development of robust, automated systems for sperm quality assessment that can be integrated into modern fertility workflows.

2. Structure of a Semen and Sperm Cell

Sperm or seminal fluid is an organic fluid that may or may not contain sperms. The male reproductive cell is the sperm, and it is secreted by the sexual glands and other sexual organs of male and can fertilize the female ova. the smaller of the gametes is known as sperm cell or spermatozoon and the more corporeal as spermatium. Human seminal fluid contains numerous different elements along with the spermatozoa: proteolytic and other enzymes and fructose are some of the elements of the human seminal fluid that increase the lives of the spermatozoa and also allow the spermatozoa to move or swim. The semen is made and its origin is in the seminal vesicle which is located in the pelvis [13].

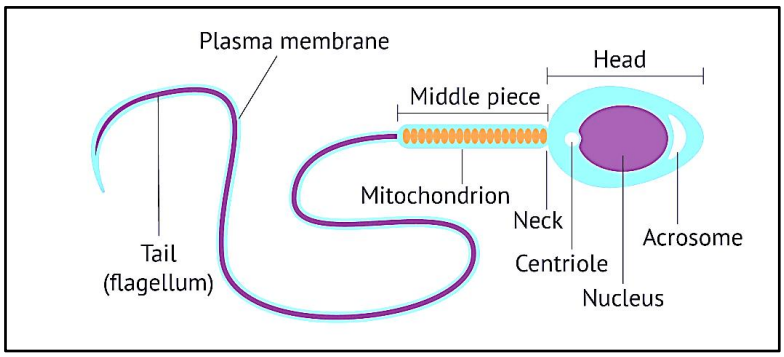

Fig (1): The Structure of a Semen [14]

3. Sperm Vitality and Vital Staining

Sperm vitality refers to the proportion of live sperm cells in a semen sample. It is especially important when motility is low, as not all immotile sperm are necessarily dead. The most common method for assessing vitality is the eosin-nigrosine staining technique:

- **Dead sperm** have damaged membranes that allow eosin dye to penetrate, staining them **red or pink**.
- **Live sperm** have intact membranes and do not absorb the dye, appearing **unstained (white)** against the dark nigrosine background.

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This test helps differentiate between live and dead sperm and is recommended when more than 40% of sperm are immotile.[15]

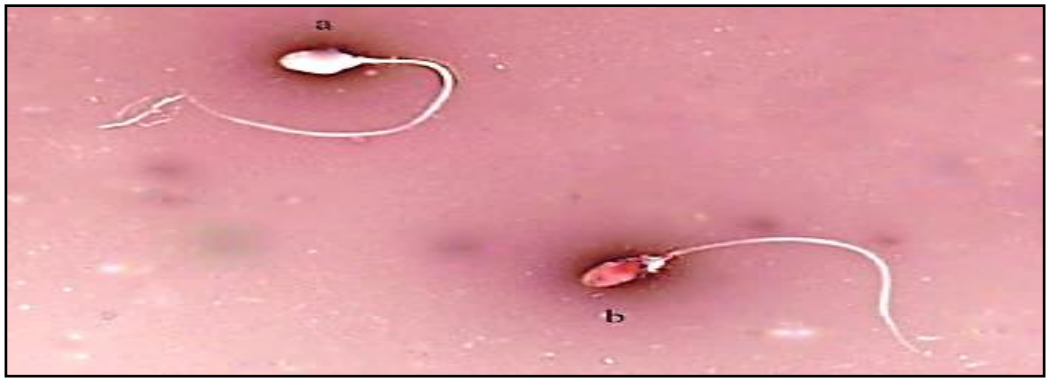

Fig (2): Assessment of human sperm viability with the aid of eosin - nigrosine staining, (a) control. unstained (white) alive spermatozoa, (b) stained (red) dead spermatozoa [16].

Distinguishing between viable and non-viable spermatozoa is central to fertility assessment. Researchers have used both physical observations and imaging methods to highlight these differences, moving from motility checks to staining and digital analysis. To support a deeper understanding of the role of live and dead spermatozoa role in fertility assessment, the following tables provide a comparative overview of live and dead spermatozoa, and focus attention on their physical attributes and imaging responses.

Table 1. Physical Characteristics of Live vs. Dead Spermatozoa

Parameter	Live Spermatozoa	Dead Spermatozoa
Motility	Active, progressive forward movement	Absent or non-progressive movement
Membrane Integrity	Intact plasma membrane	Damaged or disrupted plasma membrane
Morphology	Normal head, midpiece, and tail structure	Often distorted or fragmented structures
Metabolic Activity	High ATP production, active respiration	Reduced or absent metabolic activity
Response to Stains	Excludes vital dyes (e.g., eosin)	Takes up vital dyes due to membrane damage

Table 2. Imaging and Staining Parameters

Imaging Parameter	Live Spermatozoa	Dead Spermatozoa
Vital Staining	Unstained (membrane prevents dye entry)	Stained (dye penetrates damaged membrane)
Fluorescence Imaging	Green fluorescence with viability dyes (e.g., SYBR-14)	Red fluorescence with dead-cell dyes (e.g., propidium iodide)
Phase-Contrast Microscopy	Clear outline, refractive, dynamic movement	Darker, less refractive, immobile
Digital Image Features	High texture uniformity, elongated shape	Irregular texture, fragmented or rounded shape
Machine Learning Classification	Features cluster in “viable” category	Features cluster in “non-viable” category

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4.Research Objectives

The main objective of the proposed research is to design and test image-based tools to differentiate between live and dead spermatozoa following vital staining with the help of medical imaging and computational analysis. In particular, the following objectives are involved:

- Automated Classification: To develop an effective, automated method which can distinguish stained alive and dead sperm cells through image processing and machine learning.
- Precision and Consistency: To increase the accuracy and repeatability of sperm viability sorting over manual inspection, which will decrease operator bias and subjectivity.
- Feature Analysis: To determine and examine important morphological and color-based properties of stained sperm images that can differentiate viable and non-viable cells.
- Workflow Integration: In order to determine the possible incorporation of the suggestion into workflow of clinics or laboratories to support quicker and more effective sperm selection.

5. Experimental Procedure

The present research has a systematic methodological design to examine and isolate live and dead spermatozoa after staining based on medical imaging and computational approaches. The main steps are illustrated in the following diagram:

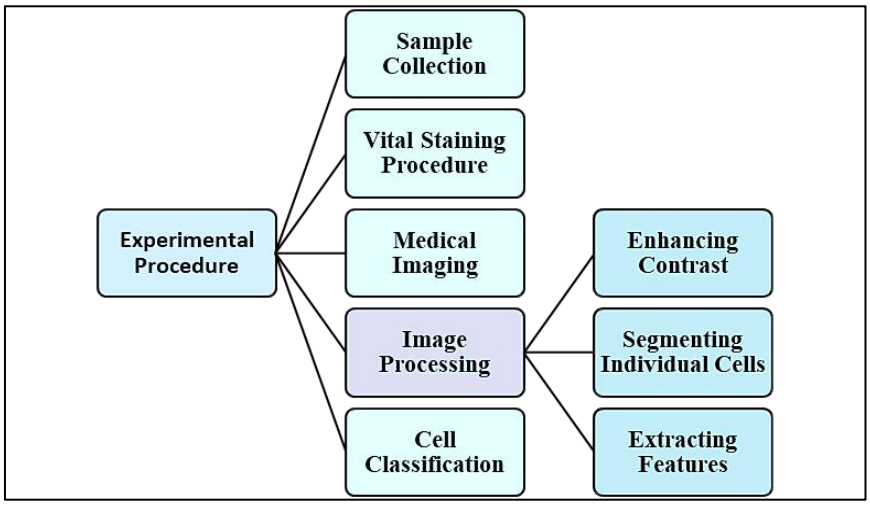

Fig (3): diagram of Experimental Procedure

6. Color Detection using MATLAB

The search to identify the optimal semen analysis technique yielded several staining techniques of sperms. None of them is however, an error free technique, and this relates mostly to the result interpretation, so we are inclined to employ color detection method in identifying the Semen volatility basing on the disparity in the living dead sperm [17]. The field of computer has a broad scope of color detection. The color detection is meant to make the difference between the objects in an image depending on their colors. With such a detection, you will have the opportunity to do any form of process in relation to that object. Similar to tracking, real color is broken down to basic color percentages whilst analyzing images in RGB space. Red (R),Green (G) and Blue (B) colors are fundamentals in this color space and all other colors may be found by mixing them up [18].

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7. Materials and Methods

The outcome of the vitality test is usually characterized as the percentage of sperm which is alive and of course the higher the percentage of living sperms the better, one of the ways of determining whether sperms are alive or not is to dip them into colored dye. A dead sperm is unable to force the dye out of its body and alive sperm can, as in the Figure.4, the sperm is stained by a pink dye known as Eosin. Those sperms that turn pink are the dead ones and those that make the dye go outside and remain clear are the live ones (light bluish green in the microscopic pictures). In this stain the nigrosin is used to enhance the shading of the background against the sperm heads, making them easier to see [19].

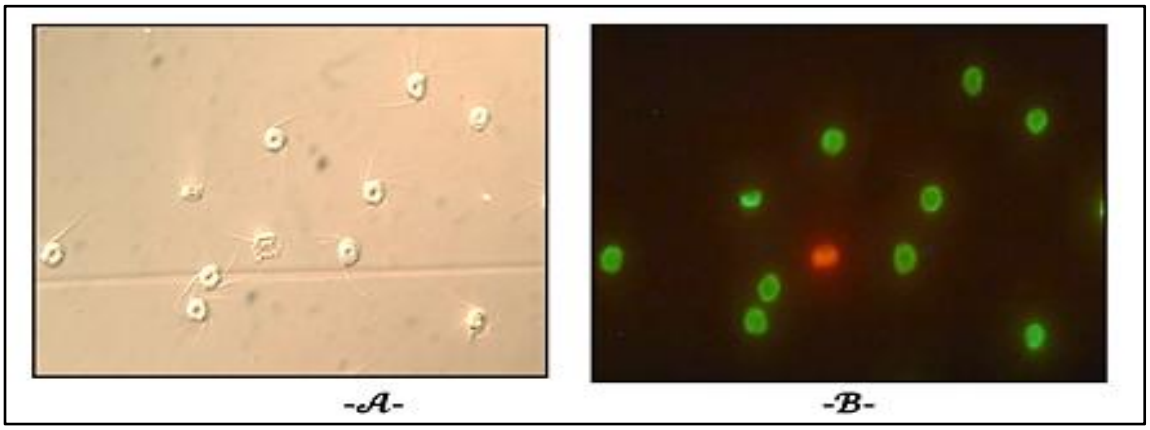

Fig (4):($\mathcal{A}$) Original image before fluoresced stain, ($\mathcal{B}$) The image after fluoresced stain.[19]

7.1 The Medical Images Under Study

The medical images used in this paper are microscopic images of seminal fluid, with different (magnification, size, and resolution) those images have been taken from different male species, where the sperms are smudged by two sorts of dye. These different images have been downloaded from different websites on the Internet.

7.2 Experimental Analysis and Discussion

The suggested algorithms have been applied to sperms vitality test Based on color detection techniques because it is the color of dead sperms to turn red and living sperm to turn green after coloration with eosin stain, the MATLAB functions given illustrate a straightforward approach to the training and testing of a color detection system. Generally, the color detection algorithm scans an image to find pixels with a particular color. The RGB color space has been employed in this program hence the color detection algorithm can be searched according to the color position and specific RGB color values.

7.3 Methodology

This section gives an algorithm to sort the alive sperm and the dead sperm on the medical images, Figure.5 gives the flowchart of the proposed algorithm and is explained in the following Steps: -

Step1:_ Image acquisition.
Step2:_ Read and display an original image.
Step3:_ Split the image into three color bands [red, green, blue].
Step4:_ Compute the threshold of each color band.
Step5:_ Amalgamate all threshold color bands.
Step6:_ Fill all image regions and holes.
Step7:_ Separate the (living & dead) sperms in Semen by color.

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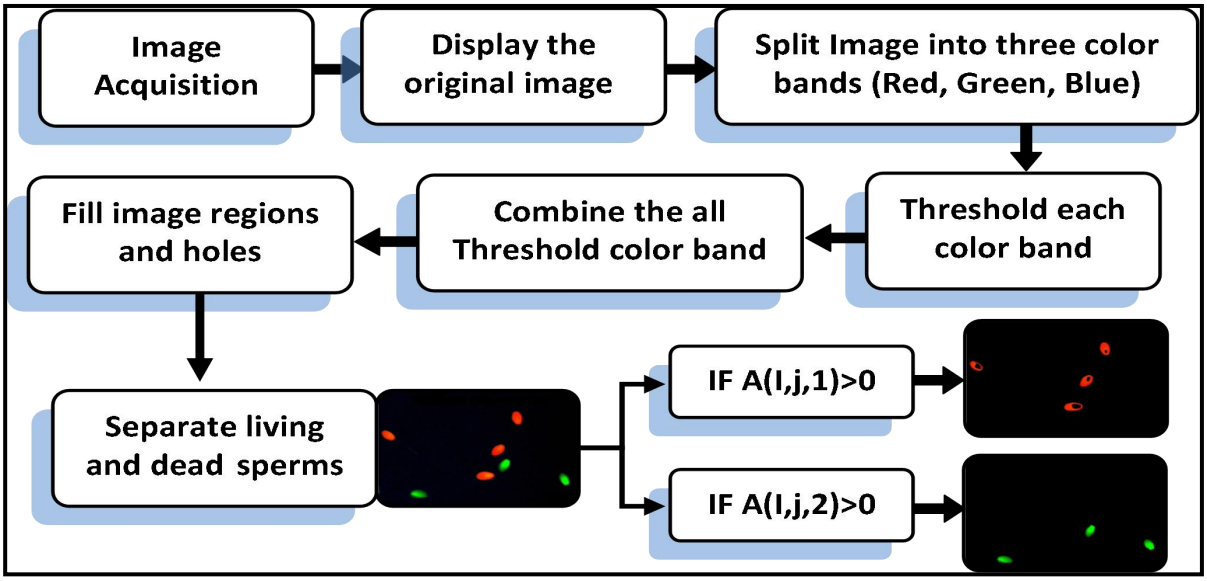
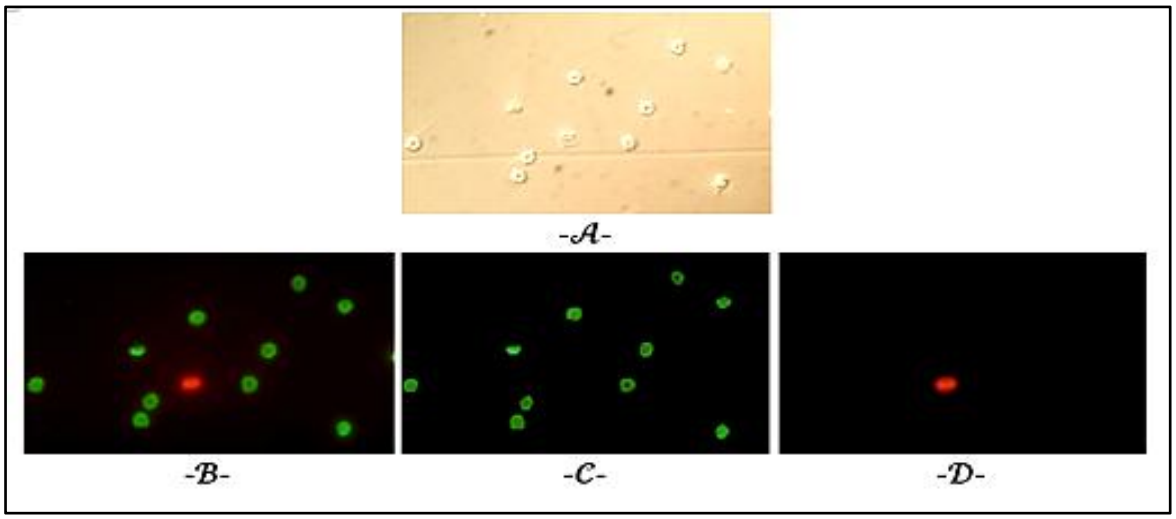


Fig. (5): Flow Chart of Algorithm

7.4 Procedure Followed and Experimental Results

The images of the sperm acquired are analyzed using image processing techniques (color detection) to determine whether the sperm in Semen sample is alive or dead by their color. To find an object of a certain color in an image. then it needs to break the image into its parts, the hue (red) and the saturation and the value color bands (red, green, blue) and calculate the thresholds and finally, generate a mask of the regions in the image that we desire to be of the color we specified, and then multiply this mask by the original image to display the image with only the color we wanted in it and all the rest blacked out. Live and dead sperm proportions of mammalian semen were easily determined, by taking advantage of the color identification methods.



Fig(6) :($\mathcal{A}$) Original image before fluoresced stain, ($\mathcal{B}$) The image after fluoresced stain, ($\mathcal{C}$) green objects represented living sperms, ($\mathcal{D}$)Red object represented dead sperm.

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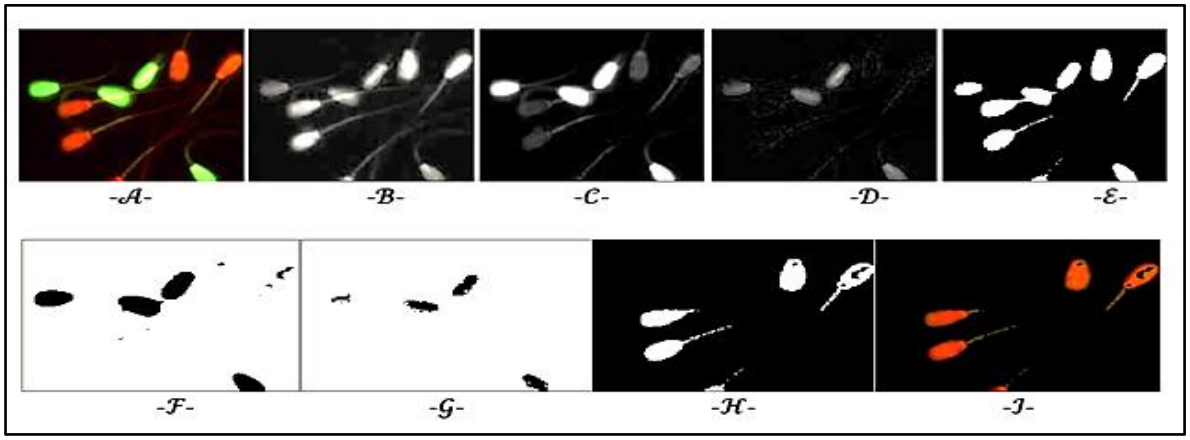
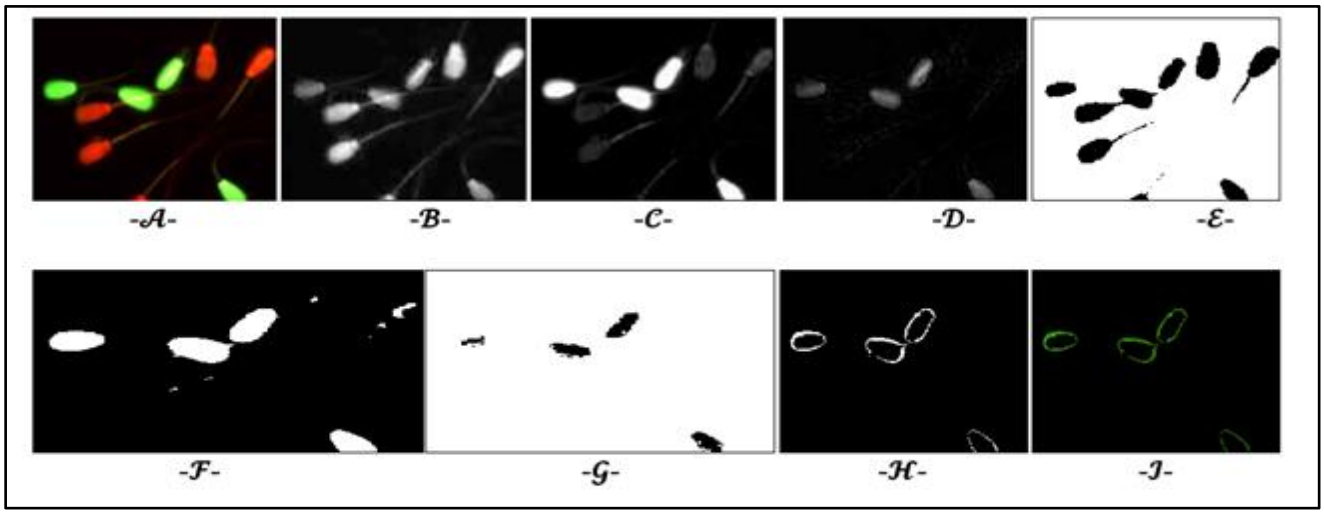


Fig (7): differentiated algorithm working scheme ($\mathcal{A}$) The Original Image [20], ($\mathcal{B}$) Red Band image,
($\mathcal{C}$) Green Band image, ($\mathcal{D}$)Blue Band image, ($\mathcal{E}$) Red mask image, ($\mathcal{F}$) Green mask image, ($\mathcal{G}$)Blue mask image, ($\mathcal{H}$)Combined mask image, (i) Red object only (dead sperm)



Fig(8): The working scheme of differentiated algorithm,($\mathcal{A}$) The Original Image [20],($\mathcal{B}$) Red Band image, ($\mathcal{C}$) Green Band image, ($\mathcal{D}$)Blue Band image,($\mathcal{E}$) Red mask image, ($\mathcal{F}$) Green mask image, ($\mathcal{G}$)Blue mask image, ($\mathcal{H}$)Combined mask image, ($\mathcal{I}$) Green object only (living sperm).

8. Conclusion

In this research, a digital algorithm was developed and implemented using MATLAB to separate live and dead sperm based on their vital staining properties. After applying eosin staining, dead sperm showed a red coloration due to the dye penetrating through their damaged membranes, while live sperm remained unstained due to their intact membranes. This algorithm was based on the analysis of digital images to effectively determine color variations in order to classify a sperm in a microscopic image efficiently and quickly. The outcomes revealed the effectiveness of the suggested technique in the automatic separation of living and dead sperm, which makes it more likely to be used in medical and diagnostic practice, specifically, the evaluation of semen quality in a laboratory. It is an algorithm that can handle numerous types of sperms samples, and it has been created as a system that gathers tools that will be applied to work in a clinic and research. Its fields of application are in the human and veterinarian clinic, scientific research, livestock production and pharmaceutical and reproductive toxicology, besides, its works in any state of hardware and

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operating-system to adapt them to the requirements of the users, as far as possible.

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