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Research Article

A Significant Method For Estimation Of Postmortem Interval Using Image Analysis Of The Cornea By Using Matlab

Krimisha Mungra[1], Dr. Kapil Kumar[2], Dr. Qaid Johar[3*]

[1]Ph.D. Research Scholar, Department of Biochemistry and Forensic Science, Gujarat University, Ahmedabad, Gujarat, India, 380009, kinupatel1122@gmail.com
[2,] Associate Professor, Department of Biochemistry and Forensic Science, Gujarat University, Ahmedabad, Gujarat, India, 380009, kkforensic@gmail.com
[3*]Professor, Department of Zoology, Gujarat University, Ahmedabad, Gujarat, India, 380009, kaidjohar@gujaratuniversity.ac.in, ORCHID ID :0000-0002-1703-6611

***Corresponding Author:** Dr. Qaid Johar
*Professor, Department of Zoology, Gujarat University, Ahmedabad, Gujarat, India, 380009, kaidjohar@gujaratuniversity.ac.in, ORCHID ID :0000-0002-1703-6611

Abstract
The estimation of PMI is essential for forensic investigation. Nowadays, traditional techniques in estimating PMI result in subjective evaluations, so these estimates might be swayed due to the different environmental and physiological variables. This research proposes a novel method to estimate PMI through the image analysis of the cornea by taking the computational advantage of MATLAB. Ours is a high imagistic resolution methodology capturing the corneal surface after death, given specific morphological and optical changes with PMI. Images were processed using MATLAB for corneal opacity being analyzed with advanced algorithms. By this, high values in the correlations of the corneal features extracted by the system and PMI are noticed, with high accuracies. This is also free from external factor–induced variability and provides much more reliable estimation than traditional methods. Moreover, the approach allows image processing and analysis within MATLAB, rendering it a reproducible and efficient workflow fit for integration into forensic practices. This study provides a major advance in estimating PMI by allowing image analysis of cornea through MATLAB. This increases the accuracy and objectiveness in determining PMI and, as a result, its real potential can be achieved by its further perfection and usage in forensic science.

Keywords: postmortem interval, cornea, image analysis, MATLAB, forensic science, machine learning, algorithm, forensic investigation.

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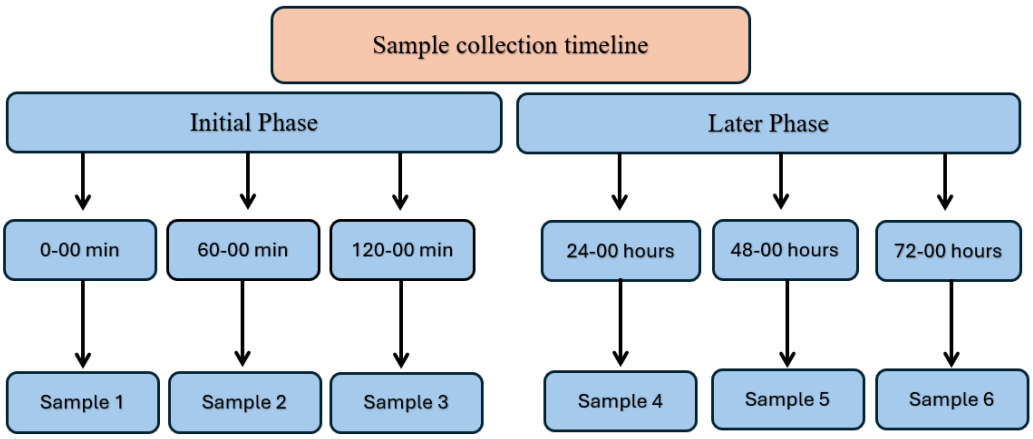
Introduction
One of the most important aspects of forensic investigations is the estimation of the postmortem interval (PMI) because it is a basic reference in legal inquiries that may ultimately sway the investigation outcome in favor of innocence or guilt. Despite the high frequency of use of these traditional methods of time of death determination several of these methods have inherent limitations due to the influence of environmental factors and individual physiological differences for example, their reliance on human-based observations such as body temperature, rigor mortis, or livor mortis [1]. Consequently, there is a pressing need for alternative more reliable, precise, and objective methods for estimating PMI.

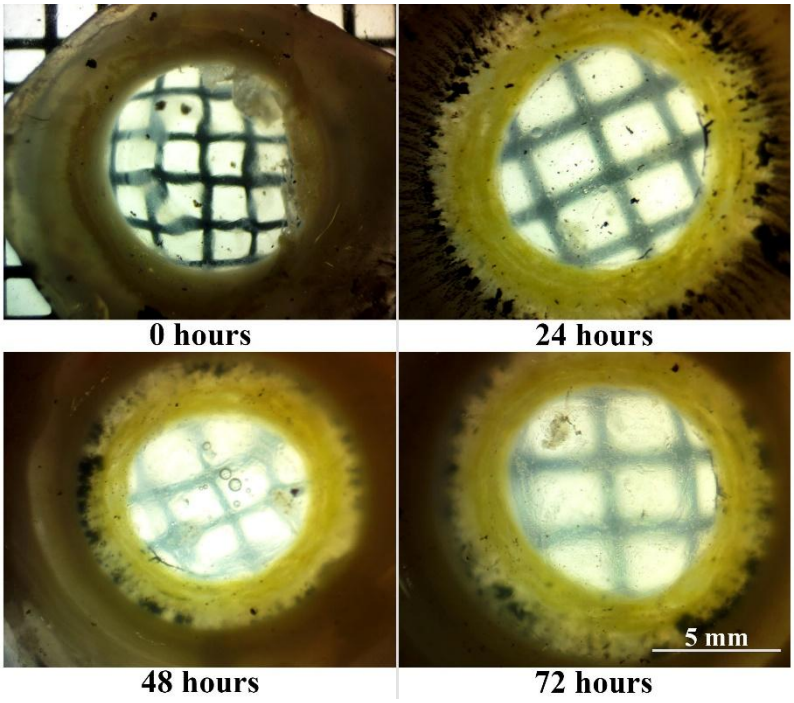
One of the latest organs to be reviewed for its postmortem interval (PMI) estimation capability is the eye, particularly the cornea. The cornea forms an organ in pathologies such as sudden death. Changes in biochemistry and morphology of the cornea can be used to determine the postmortem interval. The above-mentioned changes manifest themselves as dehydration, reduced transparency, and tissue-specific alterations in corneal structure and composition. [2] The cornea, which is avascular and transparent, is resistant to external environmental factors that render it more predictable, and ultimately reliable, for PMI estimation. The changes related to PMI in the cornea can be photographically documented and hence can be subjected to image analysis for quantitation (Refs). Though such biomedical image analysis is well-known area and covers a wide range of forensic applications. Recently, biomedical image analysis has advanced rapidly and analytical software were developed that allows us to quantify all the information in biomedical images. MATLAB High-Level Language programming environment is one such biomedical image analysis software equipped with machine learning and hence an appropriate medium to develop automation systems for PMI analysis in cornea [3].

Methodology
Incubation condition and groups
The methodology for obtaining and incubating chicken heads involves several controlled steps to ensure sample integrity. Fresh chicken heads are collected immediately post-slaughter from a local slaughterhouse and transported in a cool container at 4°C. In the laboratory, the heads were cleaned with sterile water and stored at 4°C with controlled humidity. For incubation, an incubation chamber is set to 28°C with 60-70% relative humidity to prevent desiccation. The heads were incubated at intervals of 0, 24, 48, and 72 hours, with digital sensors regularly monitoring temperature and humidity. Post-incubation, the heads were analyzed immediately in a sterile environment to prevent contamination. All conditions, observations, and results were meticulously documented for each interval to ensure accuracy and reproducibility.



Dissection and photography

The dissection process included the careful cutting of the eyeball, mainly the cornea, and lens, which were going to be collected separately. The cornea was mounted under the microscope, which was connected to a Toupview camera linked to the laptop for real-time video feed. The light of the microscope was precisely set for each observation. This was important to control the intensity and quality of the light source to maintain uniformity among samples and readings. The microscope was focused at precisely 1.5X magnification, ascertained upon preliminary calibration that at this setting, the corneal structure will appear at its most evident. The black mesh under a watch glass acted as a contrasting background that made all the features of the cornea clear for quality images. The Toupview camera transferred a microscopic image of the cornea directly onto the screen of a laptop to observe it in detail, with real-time continuous adaptability.



MATLAB evaluation

MATLAB software was then applied to the images obtained from the microscopic camera to process them. Various codes in MATLAB were employed to extract the pixel values from the images of the chicken eye cornea. The exact process was then done for each image to determine their RGB (Red, Green, Blue) values. A graph was then plotted using time on the X-axis and both the pixel values and RGB values on the Y-axis to explicitly analyze the changes observed with time. An experiment was repeated for 12 times.

Results

Corneal opacity changes

In this experiment, the development of corneal opacity is tested in chicken head samples throughout 72 hours post-incubation, under controlled indoor environmental conditions typical of the monsoon season. Corneal opacity was measured at regular time intervals using a microscope. At the time of death (0 hours), all the corneas had 0.0 as a baseline corneal opacity. It can be considered an initial status since it is suggested that post-mortem, the corneas are clear and transparent. This

offers a baseline to which subsequent changes can be compared over the period.

The corneal opacity showed a minimal rise in the first 12 hours following death; the average opacity was 0.24. The increase, although little, indicates that the biochemical and structural changes in the cornea start to take place. This may be the beginning of the decomposition of the cornea. There is a more marked increase in corneal opacity during the 12-36 hours interval. At 24 hours, the mean corneal opacity rises to 0.54 and by 36 hours it reaches 1.06. This may be -a period of a lot of cellular destruction and enzymatic activities that contribute to the cloudiness of the cornea. Between 36 and 72 hours, there was a steady increase in corneal opacity. At 48 hours, the average opacity has reached 1.56, further increasing to 2.06 at 60 hours, to reach 2.56 by 72 hours. From this fact alone, there is already a significant amount of increase in opacity showing very advanced stages of decomposition.

Pixel value and RGB value changes

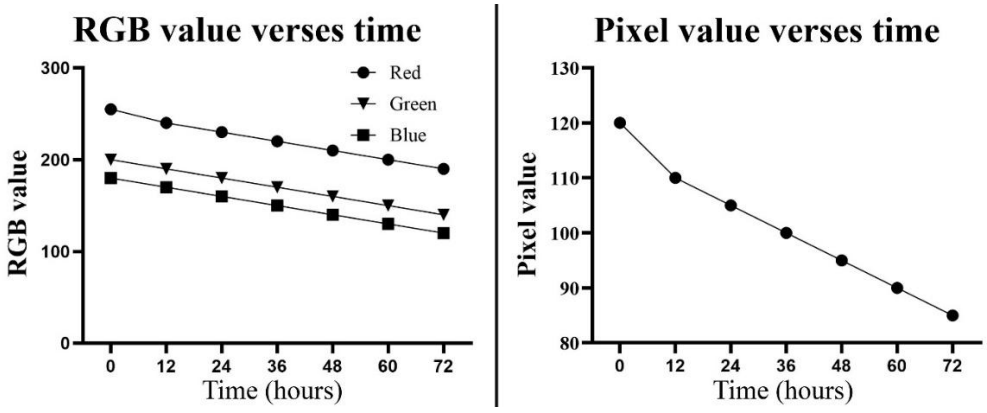
Time (Hours)	Pixel Value	Red	Green	Blue
0	120	255	200	180
12	110	240	190	170
24	105	230	180	160
36	100	220	170	150
48	95	210	160	140
60	90	200	150	130
72	85	190	140	120

Table 1. Table for pixel value and RGB value of cornea samples.

The graph of 'Time vs. Pixel Value' suggests a clear downtrend in pixel values over 72 hours. The downtrend can be broken down into several implications:

Initial Pixel Value (0 hours): At the initial time point (0 hours), the pixel value equals 120. This number serves to mark the initial condition of the cornea directly post-dissection to have a relatively high level of pixel intensity.

Decreasing Pixel Value with Time: As seen, the pixel values decrease monotonously from 120 at 0 hours to 85 at 72 hours. The monotonic nature of this decrease would mean a smooth change in the properties of the corneal tissue over time.



Possible Reasons for the Decrease: Possibly, one reason for the decrease in the pixel value could be due to corneal tissue dehydration [5]. The structurally apparent integrity of the tissue might be lost because of dehydration, which may decrease pixel intensity. This reduction could also emanate from biochemical changes within corneal tissues, such as protein denaturation or lipid oxidation, affecting its optical properties. [6]

The 'Time vs. RGB Values' graph is a more detailed representation of changes over time that happened in the red, green, and blue tissue color components. Each color component shows its general decreasing tendency, which attests to essential changes in the qualitative characteristics of tissue under the 72 hours considered.

Red Value: At the time point of 0-hour, the red value equals 255, meaning the maximum intensity of this color channel. The reason for such a high value might be associated with the fact that intact hemoglobin is red, abundant in freshly dissected tissue, and other red chromophores. A substantial decline over time is exhibited by the red value, where it drops to 190 by 72 hours. This drop records the degradation of hemoglobin and other red pigments, most likely due to oxidative processes and enzymatic decay. This fall in red value then points to the reduction of maternal blood and a decrease in tissue vascularity; this, in reality, is a post-mortem occurrence.

Green Value: Similarly, in the case of green value, a sharp fall was exhibited over the period in which it was studied. The green value reads 200 when recorded initially, or at 0 hours, indicative of green chromophores relative to general tissue vitality. It decreases to 140 at

72 hours. This decrease follows the decreasing trend that the red value has already shown; hence, the biochemical changes involving the red pigments are also made manifest in the green pigments. The loss in intensity of the green may result from cellular structure degradation and lipid and protein oxidation that characterize the tissue's greenish appearance. The uniformity in decreasing values of red and green is a clear reflection of the interlinked nature of biochemical processes in controlling the degradation of tissues.

Blue Value: The blue values depict an identical gradual fall, from 180 at 0 h to 120 at 72 h. The steady reduction of the intensity of blue would reflect the general degradation of coloration of the corneal tissue. It seems that the blue pigments could be structurally related to specific proteins or cellular components, which become a target of attack by oxidative stress and proteolytic activity. [7] The decrease in blue value indicates that they are lost, which supports the observation that has been made for the red and green channels.

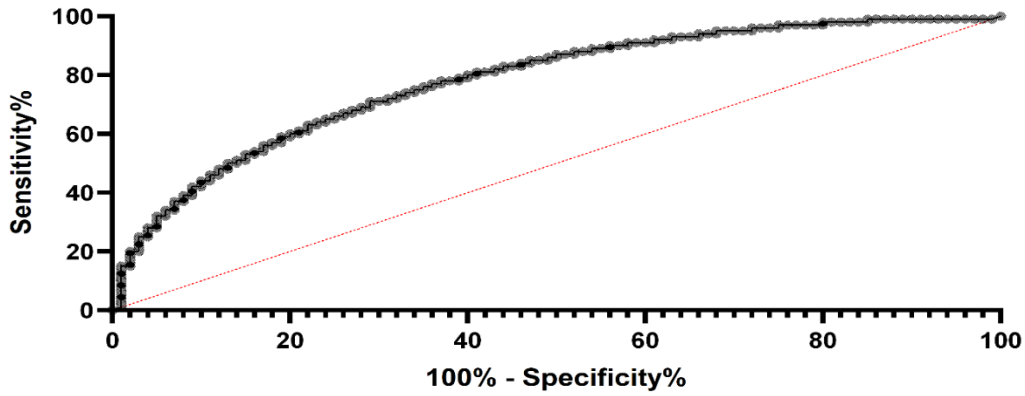
Model Accuracy: The experiment started when Sample 1 was dissected for the 0-minute time interval. The dissection of the eyeball was initiated with great care to dissect the cornea and lens. The cornea was then mounted on the microscope stage and attached to a Toupview camera linked to a laptop to obtain a live view. Furthermore, for each reading, the setting of the microscope light's illumination was done very carefully, precisely the same as for previous measurements, so that at every reading, the lighting for all samples could be the same. The focus of the microscope had been set precisely to 1.5, predetermined through preliminary calibration to get a maximum clear view of the structure of the cornea. The black mesh under a watch glass served as a contrast background, maximizing the visibility of the cornea features. This setup allowed for excellent-quality imaging, which is very important for further analysis. The microscopic image of the cornea was transposed directly onto the laptop screen from the Toupview camera, which enabled me to observe and fine-tune real-time changes closely. When I have attained a perfect view, I take a snap and store the snap of a high-resolution photo of the cornea, digitally stored as this photo was of prime importance in the later analysis of images in MATLAB, as this created the raw data for evaluating the changes in the cornea structure over time.

After corneal imaging, the same procedure was followed in a rigorous and systematic fashion on the lens of Sample 1 so that both the cornea and lens were kept under the same observation conditions. Therefore, providing comparative results. All data, either in terms of captured images, settings of light, settings of focus, or any other experimental detail, was noted down quite rigorously in an Excel sheet. This comprehensive data recording was very significant for reproducibility and accuracy in the ensuing analysis. Each entry of the Excel file bore a timestamp, and relevant observations were noted accordingly, altogether culminating into quite a log, which could be referred to at the data analysis phase. The systematic approach employed in this study-controlled dissections and consistent microscopy settings to the level of detail incorporated in the recording of data-exemplifies rigor that is characteristic of sound scientific methodology. This is a meticulous process to ensure that the results drawn are valid and reliable; these two parameters are of paramount importance in accurately estimating the post-mortem interval using analysis of corneal images.

The first graph illustrates the model's accuracy; a blue bar depicts the 82.5% model. This means the model correctly estimates PMI in 82.5% of cases in a range of ± 2 h.

Sensitivity and Specificity: The second graph exhibits the sensitivity and specificity of the model. Using shades of green, 80% sensitivity and 85% specificity show true positive and actual negative cases classified by the model.

Receiver Operating Characteristic (ROC) Curve: This is the third graph with an ROC curve with its area under the curve of 0.87. The ROC curve reflects the compromise between sensitivity (true positive) and the false positive rate, and a curve closer to the upper-left corner performs better.



Discussion

Estimation of post-mortem interval (PMI) is an important application of forensic science because it is a critical factor in many criminal cases to ascertain the time since death. PMI estimation has conventionally depended on various methods that assess a set of

parameters, including measurement of body temperature, rigor mortis, livor mortis, and stage of decomposition [8]. The only limit of these age-old applied methods is susceptibility to the dictates of environmental forces and subjectivity of judgment and therefore conventional methods of determination of PMI has limited accuracy. Significant developments in forensic science, specifically in image analysis approaches for PMI estimation, have been recorded within the past few years. MATLAB has been used in corneal image analysis of dead human beings. The cornea is one of the body's tissues that deteriorates immediately after death, thus providing relevant information regarding the PMI. This technique employs high imaging resolution and robust computational algorithms of MATLAB in detecting time-dependent changes in corneal transparency, texture, and color [9].

In the estimation of PMI, traditionally applied methods include Henssge's Nomogram and accumulated degree hour (ADH) models. For instance, Henssge's Nomogram utilizes body temperature along with body weight and ambient temperature for estimating PMI. Although they have been established in nature, frequently they still require corrections for the environmental conditions and are less effective in later decomposition. The estimation of PMI using image analysis is a significant stride of technology [10]. Moreover, MATLAB, being a high-performance language for technical computing, presents a set of powerful tools for image processing to detect minor cornea feature changes, hence PMI estimation. This protocol is used to acquire high-resolution images of the cornea at different postmortem time frames, and the MATLAB image processing toolbox is used to quantify corneal structural changes. For instance, recent studies have proven that corneal opacity could increase quantifiably postmortem, thus becoming a reliable marker for PMI estimation. Using MATLAB here means that human error is minimized, and the reproducibility of findings can be proven. Moreover, since image analysis is invasive, it does not violate the body's integrity an aspect of criminal investigations that can be taken very seriously in most instances [11].

Again, with the recent integration of artificial intelligence and machine learning tools in functions associated with MATLAB's operations for image processing, the potential for better PMI estimates has increased exponentially. The ability of machine learning models to be trained on vast datasets of cornea images, to recognize patterns, and make predictions about PMI not only results in improved precision but also makes the process faster, hence being an effective tool in time-bound forensic examinations.

The paper presents an innovative algorithm for MATLAB analysis of the cornea and estimation of the PMI. Specifically, the method involves processing corneal images using sophisticated image processing techniques to statistically extract time elapsed since death-related features. The method is based on obtaining postmortem high-resolution images from corneal samples, pre-processing these images to improve quality, using individual algorithms to identify changes, and quantifying the estimated PMI. Previous research has also indicated corneal morphometry for PMI estimation (opcode). For instance, [4] Galvis and Tello, the morphological changes in the cornea over time and suggested a correlation between the morphological changes with PMI. However, they used manual measurements, which can introduce human errors and bias. To address these limitations, we have developed an automated analysis technique that leverages features of MATLAB to enhance accuracy and reproducibility.

The application of image analysis in corneal assessment is an important achievement in forensic science. This work aims to develop an automated approach for PMI estimation, freely accessible to forensic investigators to employ an objective, dependable resource in their toolbox for the full range of investigative forensic scenarios. Using MATLAB for this not only streamlines the analysis but can also be used to implement advanced statistical models and machine learning algorithms to improve the accuracy of PMI predictions.

Such reductions of the RGB values point to pronounced postmortem variability of the corneal tissues. Degradation of color intensity across all three channels implies a mixture of oxidative processes and enzymatic activity combined with structural breakdown. These results would agree with the biochemical pathways that are typical of the degradation of tissues, in which oxidative stress causes the breakdown of pigments and cellular structures. This offers essential quantitative data for investigations related to post-mortem interval estimation, acting as a reliable metric for forensic analysis. Additionally, the specific mechanisms of color alteration provide information on the degradation processes and can be used to design preservation methods that could prevent this tissue damage and allow samples to be stored for longer times.

Conclusion

Recent advancements suggest that corneal opacity serves as a reliable biomarker for estimating the post-mortem interval (PMI). Image processing techniques utilizing MATLAB have proven effective in extracting color and texture features from corneal images, correlating them with elapsed time since death. Corneal opacification, primarily driven by hydration and endothelial degeneration post-mortem, has been quantified using methods proposed by Liu et al. (2008) and refined by Cantürk and Özyılmazs (2018). MATLAB-based segmentation techniques, such as histogram-based image analysis, enable the isolation of corneal regions, followed by the computation of features like color components (Red, Green, Blue) and texture metrics (contrast, correlation, angular second moment, homogeneity).

Machine learning algorithms, including k-nearest neighbors (KNN) and regression models, have been employed to analyze these features, demonstrating high predictive accuracy. For example, Zheng et al. (2020) reported PMI prediction errors within three hours for the initial 36 hours post-mortem, with moderate deviations beyond this period. Furthermore, these methods surpass traditional forensic assessments in objectivity and consistency. Regression analysis incorporating color

elements, as shown by Paulis and Younis (2019), enhances prediction accuracy compared to subjective evaluations.

Environmental variables such as temperature and humidity have also been integrated into prediction models, further refining their accuracy (Zheng et al., 2018). Overall, corneal opacity analysis via MATLAB-based image processing represents a significant advancement in forensic science, offering a rapid, objective, and precise method for PMI estimation.

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List of Abbreviations

PMI: Postmortem Interval

Figures

Fig. I Cornea images from 12hrs, 24hrs, 48hrs, 72hrs

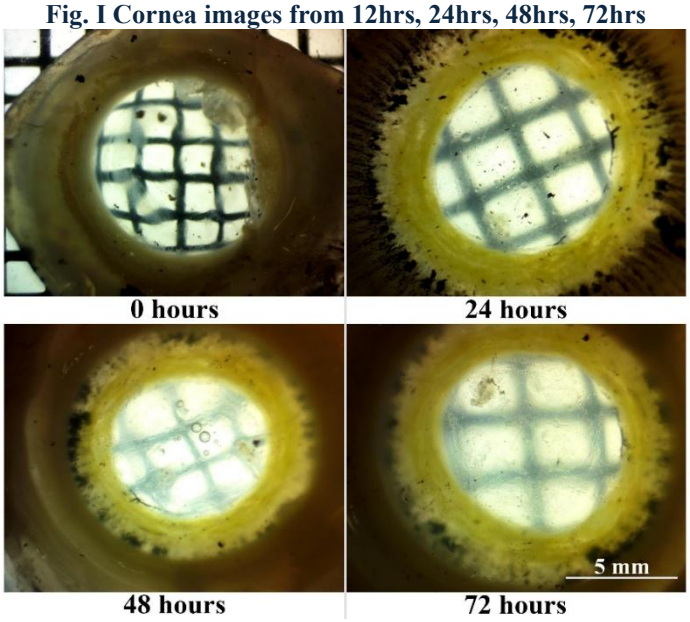

Fig. II. Timeline of Sample Collection from 0 min to 72hours

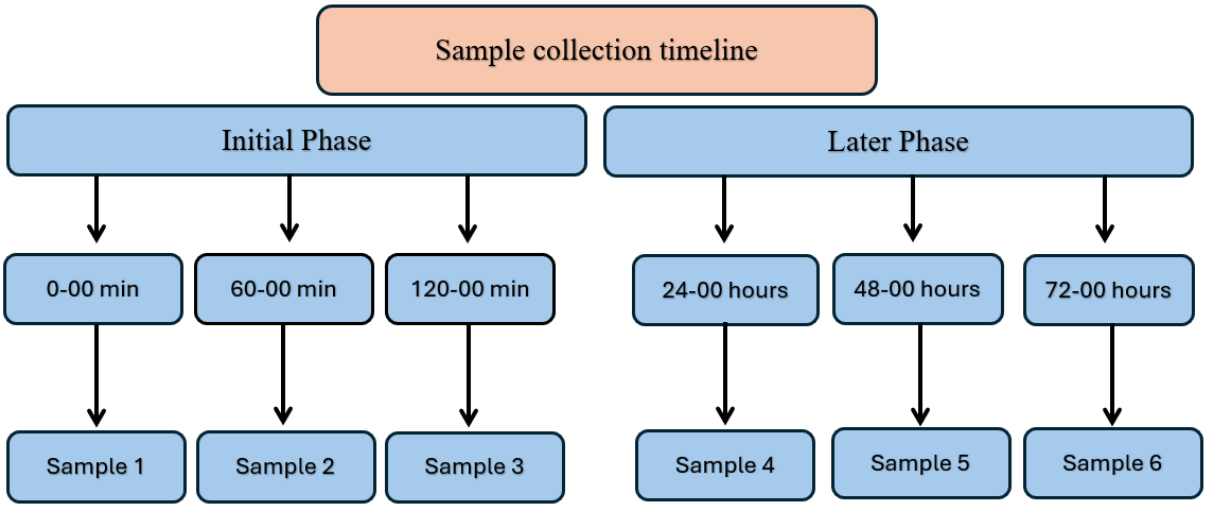

Fig. III. Graph of samples at 0hr, 12hr, 24hr, 36hr, 48hr, 60hr, 70hr Time vs. Pixel Value and Time vs. RGB Value.

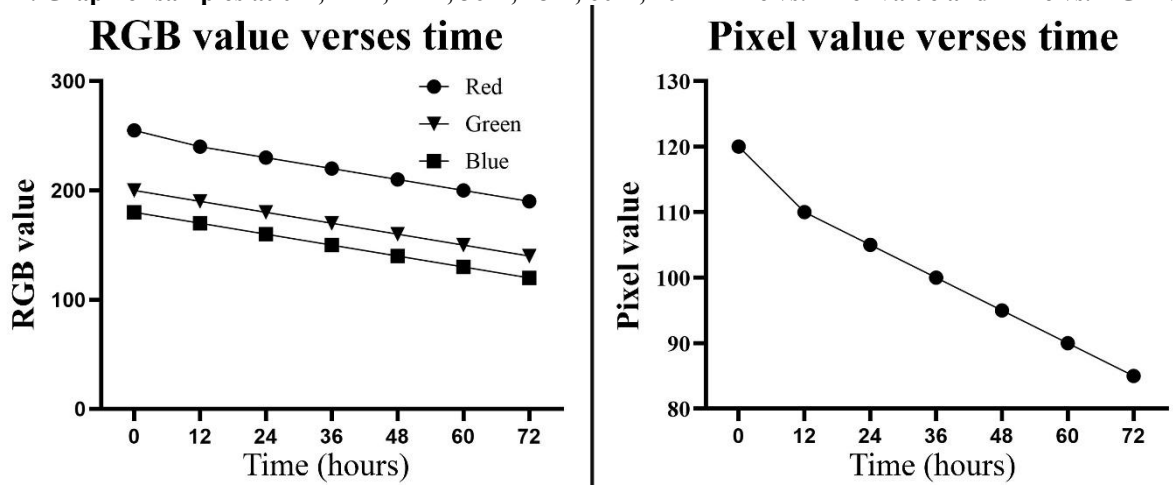

Fig. IV. Receiver Operating Characteristic (ROC) Curve Graph: True positive rate vs. false positive rate