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Biophotomicrograph, an Alternative Technique for Measuring Micro-Sized Biological Objects

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Abstract:- Tadulako University and the Polytechnic are illustrative instances of higher education establishments in Palu City that are consistently enhancing the capabilities and expertise of their faculty members, equipping them with the necessary qualifications to confront the ever-evolving challenges posed by advancements in science and technology, particularly in the domain of laboratory research. One essential skill he must acquire is proficiency in employing biophotomicrograph methods. The implementation of this technique is crucial for study programs that integrate advocacy and academic education in the laboratory domain. Its extensive and frequent utilization by students and instructors is highly beneficial for their engagement in practical and analytical activities, as well as research endeavors in the areas of health, microtechnics, micromaterials, and micropreparation. Based on the current observations and debates, it appears that these research institutes do not acknowledge the validity or significance of biophotomicrographs. Hence, the implementation of this technical training activity is deemed highly essential. The aforementioned activity was conducted over a span of two consecutive days, specifically from August 9th to August 10th in the year 2023. The activity encompassed a two-day session comprising theoretical instruction on the fundamental principles of biophotomicrograph methods, followed by a practical session involving the measurement, capture, and analysis of microscopic pictures. In practical endeavors, photomicrograph modification techniques are utilized to facilitate various procedures. Photomicrographs are generated by affixing a smartphone to the eyepiece while manually grasping the smartphone. The calibration procedure was thereafter conducted with a micrometer measuring 10 µm in size. Subsequently, proceed with the installation of the ocular micrometer within the eyepiece. The scale that becomes visible is thereafter aligned with both the ocular micrometer scale and the objective micrometer scale. The outcomes of the ocular micrometer calibration are afterwards employed for the direct or indirect measurement of microscopic entities. This is achieved by initially capturing images using a smartphone and subsequently quantifying them with the assistance of the ImageJ software. Consequently, the aforementioned endeavor successfully accomplished its objective of enhancing the comprehension, attitudes, and competencies of educational personnel in relation to the biophotomicrograph technology. Additionally, it yielded a refined version of a photomicrograph prototype that had the capability to measure micro-objects in accordance with global benchmarks.

Keywords:- *Biophotomicrograph, Imagej, Micrometer, Microscope*

I. INTRODUCTION

The biophotomicrograph technique refers to a method employed to capture images or photographs of items that are microscopic or micro-sized by utilizing a microscope. These objects are subsequently measured using a micrometer and subjected to analysis with the aid of software tools like ImageJ [1]–[6]. To date, this methodology has been implemented via digital photomicrographs obtained from a microscope equipped with an integrated camera and computer system. [7]–[12]. Nevertheless, due to the exorbitant cost associated with such equipment, its utilization among researchers and academics is seldom. Currently, there is a lack of laboratories at educational institutions such as Tadulako University, STIFA, and Cenderawasih Polytechnic. The demand for micrographs is of utmost importance in several disciplines of study, including plant anatomy, animal anatomy, micro-materials, particle analysis, blood analysis, microbiological analysis, and other microtechnics.

The unavailability of biophotomicrograph equipment due to different factors poses a significant issue that necessitates attention. One potential approach that can be employed is the utilization of technical tools, namely a smartphone and a micrometer attached to a microscope.[9], [13]–[15]. Based on a substantial period of observation and analysis, it has been determined that the aforementioned modification tool exhibits a high degree of realism, characterized by enhanced measurement precision and commendable image quality. The quality of images is significantly influenced by the type of smartphone camera employed [1], [3]–[6], [10], [11], [14], [16]. Similarly, the quality of the microscope's lens is a determining factor in its overall quality [1], [13], [14]. The aforementioned equipment is readily accessible inside the laboratory setting. In the interim, it is possible to get micrometers and smartphone holders from an online laboratory store at a cost that is deemed fair. Therefore, the implementation of this suggested biophotomicrograph is highly feasible.

The demand for biophotomicrographs among students and lecturers for the purposes of practicum and research is on the rise. There is a significant number of students at

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Palu's educational institutions who actively engage in laboratory practicums. There exists a considerable population of students who employ the biophotomicrograph technique to fulfill their final assignment requirements. Similarly, a substantial number of lecturers engage in laboratory research employing the biophotomicrograph approach. Presently, there is a lack of proficiency and accuracy in the use of biophotomicrograph techniques by both students and lecturers engaged in practicum and research activities. Photographs are captured using improvised mobile devices without consideration for their quality or adherence to rigorous accuracy, so failing to fulfill the criteria set by scientific standards. Scientifically acceptable images possess high picture quality, including a scale bar for reference, and are captured using properly calibrated equipment [5], [17]. The scale bar depicted in the image should adhere to scientific principles by accurately reflecting the measurement and calibration outcomes, specifically employing a micrometer [3], [17], [18].

The micrometer is comprised of two components: the objective micrometer, positioned on the object table of the microscope, and the ocular micrometer, located within the eyepiece of the microscope [19]–[21]. The objective micrometer is composed of glass and takes the form of a slide or item consisting of four rectangular shapes. It incorporates an engraved scale of certain dimensions. In general, the sculpture has ten prominent scales, with each scale measuring 0.1 millimeters or 100 micrometers. Each of these macroscopic sizes is further subdivided into ten smaller scales, with a measurement of 0.01 mm or 10 μm [21], [22].

In addition to the objective micrometer, another type of micrometer commonly used in microscopy is the ocular micrometer. Unlike the objective micrometer, the ocular micrometer is constructed from glass and takes the shape of a circular disc with a diameter equivalent to that of the microscope's eyepiece. The ocular micrometer contains an etched scale as well. The determination of the scale on the ocular micrometer involves calibrating it by alignment with the objective micrometer on the microscope.[19]. The ocular scale, in essence, is a numerical scale that encompasses a range from 1 to 100. The equidistance between the lines is consistent, but with an undetermined value. The size of the ocular scale remains constant regardless of changes in magnification, but the size of the objective scale varies with changes in magnification. Consequently, the process of calibration is conducted in order to establish the value of the ocular scale by comparing it with the objective scale at various magnifications. The ocular micrometer has been calibrated using a standardized method, enabling precise measurement of a specimen rather than relying on approximations [13], [14], [23], [24].

Calibration, as defined by ISO/IEC Guide 17025:2005 and the Vocabulary of International Metrology (VIM), encompasses a set of procedures aimed at establishing a correlation between the measured value indicated by a measuring instrument or system, or the value represented by a measuring material, and the known values associated with the measured quantity. These activities are conducted under specific conditions. Calibration is an essential process that involves assessing the accuracy of measuring devices and materials by comparing them to traceable measuring standards, national standards for units of measure, and/or international standards [11], [17].

The process of calibration is conducted in order to ascertain the precise dimensions of a scale by utilizing the ocular micrometer. The process involves the placement of an ocular micrometer within the eyepiece of the microscope, while the objective micrometer is positioned on the microscope table. The alignment of the two scale lines on a low-power microscope, such as a 10X objective lens, is achieved by adjusting the objective micrometer. This adjustment ensures that two identical scale lines are observed between the ocular micrometer scale and the objective micrometer scale. The calibration procedure for objective lenses with increased power is conducted in a similar manner [19], [21], [22].

The utilization of a calibrated and sized ocular micrometer enables the measurement of an item observed through a microscope. The determination of an object or specimen's dimensions can be achieved by quantifying the number of scales that span its length. The process of measurement should commence by utilizing a low-power objective lens. The size of the ocular scale remains constant regardless of changes in magnification or objective lens, but the size of the objective scale is subject to change when there are alterations in magnification or objective lens. Hence, the perceived magnitude of the object micrometer will vary in accordance with the chosen magnification or object lens. Consequently, the process of calibration is conducted in order to establish the value of the ocular scale by comparing it with the objective scale at various magnifications. The ocular micrometer has been calibrated to a recognized standard, enabling precise measurement of a specimen rather than mere estimation [19], [20].

II. METODE

The execution of tasks performed by student helpers and professors. The implementation phase will be scheduled for a duration of two days, commencing in the morning at 14:00 and concluding at 17:00. Greetings, I hope this message finds you well. The training will be conducted by presenters representing several departments. The responsibilities of the presenters will be divided, with one designated as the core presenter responsible for delivering the essential content, while the other presenters will be assigned the role of supporting and guiding participants in the practical use of photomicrographs. The presenters utilize smartphone media connected to computers and LCDs to facilitate the delivery of the core topic, hence enhancing trainees' comprehension and application of the content. Supplementary instructional content will be made available to users in the form of a movie demonstrating the proper utilization of a photomicrograph. The videos will be distributed to the attendees or made available for download on the given website.

The training program will commence with an introduction to the theoretical aspects of utilizing the photomicrograph technique. Subsequently, participants will be instructed on the procedure for affixing a smartphone to a microscope, accomplished by securely positioning it within the ocular lens. Subsequently, proceed to place the ocular micrometer into the ocular lens compartment, while placing the objective micrometer onto the microscope table. Subsequently, the individuals had training in the calibration of the ocular micrometer in conjunction with the objective micrometer.

This inquiry pertains to the process of calibration utilizing a calibration slide possessing a dimension of 2000 µm. The ImageJ software is launched, and the calibration slide is positioned on the microscope stage, which is linked to the computer. The slide is placed within the field of view of the objective lens, which has a magnification of 4x10. The picture is brought into focus until the object's image is observed as a circular shape on the monitor screen. Next, the user clicks on the capture menu and proceeds to pick the option for manual capture. The storage of images is often done in the JPEG file format. Subsequently, the software application ImageJ is launched, and the photographs that have been previously saved are chosen. Subsequently, the measure is activated by means of a click, resulting in the selection of the line. The image is captured by clicking from one end to the other end of the circle while simultaneously dragging, in order to determine the diameter of the circle. Ensure that the dimensions are precisely 2000 µm. In the event that the measurement has not yet attained a value of 2000 µm, the user is advised to navigate to the calibration menu. Subsequently, the user should proceed to access the calibration table and subsequently choose for the use of the 4 X objective lens.

The calibration slide is substituted with the specimen of interest, namely the preserved preparation, in order to quantify the dimensions of the cells, including their length and breadth, as well as the diameter of the preserved cells. Next, locate the shadow of the item on the most distinct microscopic specimen using a 10X magnification. Proceed to manually take the image and save it in the .jpg format. Following this, access the automated image menu and choose the aforementioned image as the object detected in the microscopic preparations. Next, choose the measure line tool and proceed to click on the desired cell in order to determine its length, breadth, and nucleus dimensions. To obtain the length, breadth, and diameter values, it is necessary to perform a dragging action from the end of the cell to the base of the cell.

During the subsequent phase, the participants had training in the utilization of a calibrated ocular micrometer for the purpose of measuring tiny entities. The concluding phase of the training encompasses the utilization of a smartphone for capturing photographs, the process of saving these images, and the application of a scale bar or tags to images through the use of the ImageJ software.

III. RESULTS AND DISCUSSION

The community service initiatives have effectively accomplished their stated objective of augmenting the knowledge, attitudes, and skill of the teaching personnel with regards to the biophotomicrograph technology. The use of biophotomicrograph technology is a very uncomplicated process that may be utilized in many educational settings, including institutes of higher learning. The recently created technology seems to have successfully substituted the photomicrograph equipment in the factory's continuous manufacturing operations. The participants are initially provided with an introduction to the essential characteristics of the microscope. This includes an explanation of its operating components and an overview of the many types of microscopes, as seen in Figure 1.

Fig 1 Functional Parts and Types of Microscopes.

Proficiency in microscopy is highly advantageous for educational purposes, scientific investigations, and the examination of diminutive entities. Consequently, participants anticipate possessing this capability, particularly for the purpose of observing micro-objects throughout the processes of learning and study. Specifically, those enrolled in the Medical Laboratory Technology Study Program have a strong desire and necessity to familiarize themselves with the practice of studying microscopic entities using a microscope (see Figure 2).

Fig 2 Observation of Micro-Objects using Monocular and Binocular Microscopes.

Ideally, the observation of micro-objects under a microscope does not occur by direct visual inspection, but rather through the utilization of a screen, such as a computer screen, for viewing purposes. This approach offers simplicity, comprehensiveness, convenience, and well-being. As a result, the utilization of computer-connected microscopes has become prevalent (Figure 3).

Fig 3 Digital Microscope

The cost of digital microscopes is significantly higher in comparison to conventional microscopes, hence restricting their utilization only to organizations endowed with substantial financial resources. Educational and research institutes with little financial resources continue to possess scarce funding opportunities. Hence, the integration of innovative and creative approaches is imperative in the endeavor to substitute digital microscopes with conventional ones through the incorporation of supplementary equipment that emulates the functionalities of a digital microscope. The digital microscope is modified by the incorporation of a camera or smartphone into the eyepiece, as seen in Figure 4.

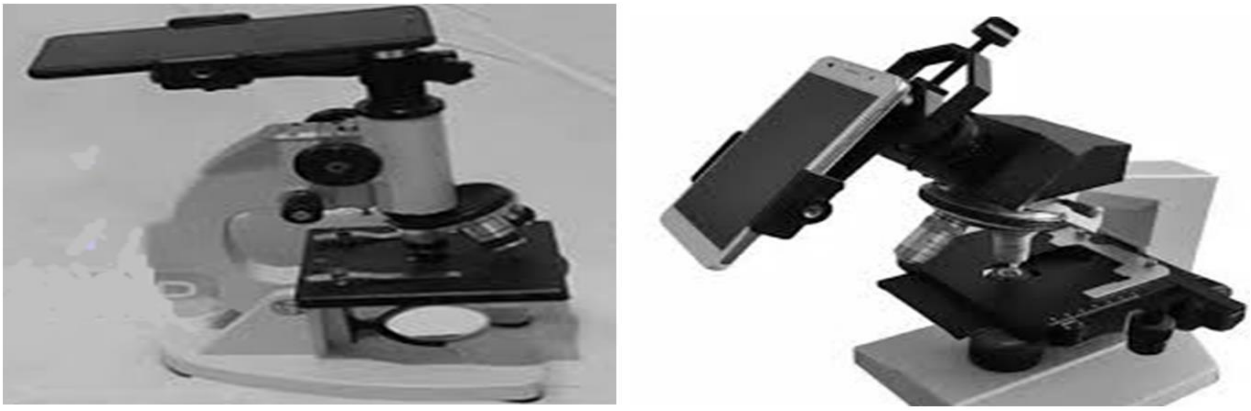

Fig 4 Adding a Cellphone above the Eyepiece Lens, Part of the Microscope.

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The integration of a smartphone with a microscope offers significant benefits for observation purposes, as it eliminates the need for direct visual inspection of micro-objects within the microscope. Instead, one may conveniently see these items through the screen of the cellphone. Indeed, the act of capturing visual imagery by shooting or recording is feasible. However, it is vital to provide careful consideration to these alterations since the use of a digital microscope may hinder the ability to get precise measurements. This limitation can be mitigated by employing either an ocular micrometer or an object micrometer, either in conjunction or independently.

The concurrent utilization of the ocular micrometer in conjunction with the objective micrometer is employed to directly measure objects. The initial step involves the placement of an ocular micrometer into the eyepiece tube. The installation process involves the initial step of opening the eyepiece cover, followed by a rotational movement. Subsequently, remove the outermost lens, which has a concave-convex shape. The removal of the ring surrounding the lens was undertaken to streamline the procedure for extracting a double convex lens. The ocular micrometer is inserted by applying pressure to the region of the tube where the eyepiece is situated. The placement of a double convex lens occurs subsequent to achieving an optimal location within the eyepiece tube, characterized by a horizontal orientation and absence of tilting. The cautious installation of this lens is necessary due to its frequent oblique positioning. Consequently, the act of inserting is accomplished by the process of aligning the tube and maintaining the lens position in a parallel manner. Subsequently, the double convex lens is gradually displaced inward until it reaches a point of cessation in its movement. Subsequently, proceed to don the ring and convex-concave lens. Ultimately, the cover for the eyepiece lens is reassembled.

The calibration procedure is conducted in order to establish traceability for a given measurement. Calibration refers to the process of ensuring that a measuring instrument's accuracy aligns with its intended design specifications. The process of calibration involves the comparison of a standard that is connected to either national or international standards or certified materials. Calibration plays a crucial role in providing valuable support for the observed data, so enabling the outcomes of these observations to be adequately justified. Moreover, the process of calibration enables the determination of the magnitude of the discrepancy between the measured quantity and the true value.

The calibration technique involves the comparison of the two lines on the scale of the ocular lens with the two lines on the scale of the objective lens, ensuring that they are parallel. The initial step involves seeing the alignment of the chosen lines on the scale of the two micrometers. This observation is conducted using the lowest objective lens available, such as a 4X magnification objective. The obtained results indicate the presence of two parallel lines, as seen in Figure 5, while employing a 4X objective lens. According to the findings presented in Figure 5, it can be observed that the two ocular micrometer lines exhibit parallel alignment with the five objective micrometer lines. Therefore, one ocular line is equivalent to 25 micrometers, calculated as 2/5 multiplied by 10. The multiplication by a factor of 10 is necessary due to the utilization of an objective micrometer with a division value of 0.01 mm, which is equivalent to 10 um.

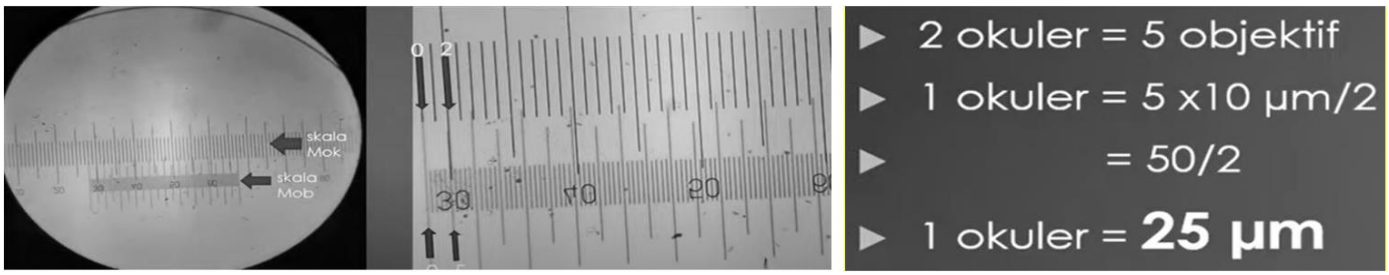

Fig 5 Calibration of the Ocular Micrometer to the Objective Micrometer when Observing using a 4X Objective Lens.

Observing using an objective lens at a magnification of 10X yields varying outcomes when calibrating the ocular micrometer to the objective micrometer. The experimental findings yielded a total of ten ocular micrometer lines, which corresponded to the measurement of a ten micrometer objective. This implies that the measurement represented by a single ocular micrometer line is equivalent to that of a single objective micrometer line. The equivalence between an objective micrometer line and 10 microns or micrometers is established, and similarly, an ocular micrometer line is equivalent to 10 micrometers (Figure 6).

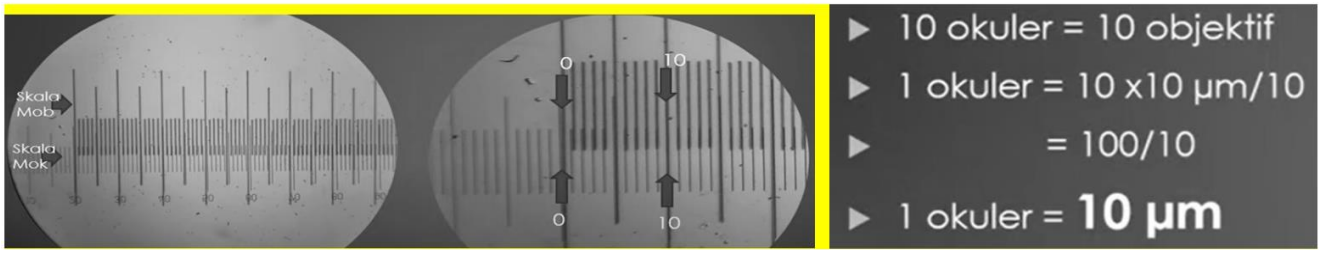

Fig 6 Calibration of the Ocular Micrometer to the Objective Micrometer when Observing using a 10X Objective Lens.

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The calibration process of the two micrometers was conducted by employing an objective lens with a magnification factor of 40. The results indicate that one line on the ocular micrometer corresponds to a measurement of 2.5 micrometers. The results presented in this study were derived from the alignment of two lines on two scales of two micrometers, specifically the ocular micrometer and the objective micrometer, as seen in Figure 7.

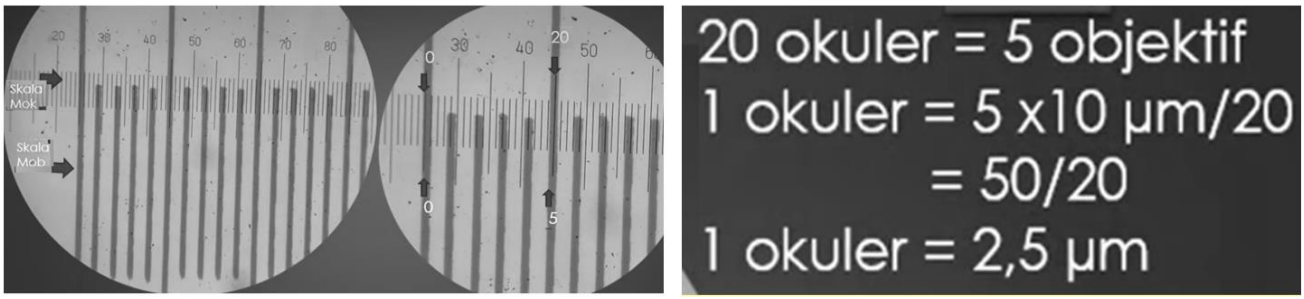

Fig 7 Calibration of the Ocular Micrometer to the Objective Micrometer when Observing using a 40X Objective Lens.

The process of calibration was conducted by employing an objective lens set at a magnification level of 100x, resulting in the acquisition of an ocular micrometer line measuring 0.9 um. The aforementioned findings were acquired by the process of aligning two lines of the ocular micrometer with a span of 22 lines, in conjunction with two lines on the objective micrometer. The procedure and computations are visually depicted in Figure 8.

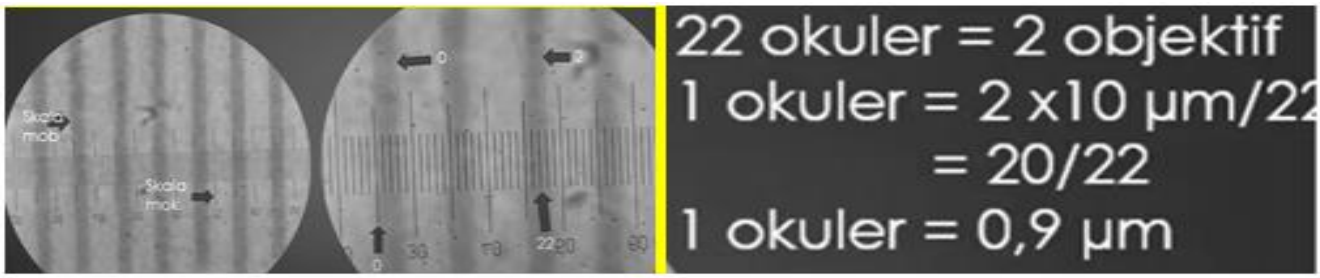

Fig 8 Calibration of the Ocular Micrometer to the Objective Micrometer when Observing using a 40X Objective Lens.

The calibration procedure holds significant importance prior to conducting measurements or capturing images. Once the calibration process has been completed, the subsequent procedure involves the installation of an image capture device in the form of a mobile phone. An auxiliary tool was utilized to include mobile functionality into the microscope. The installation of an adapter, a device used to mount a mobile phone onto the ocular lens, is performed subsequent to the installation of the ocular micrometer. The cellphone is positioned in a manner that allows the picture of the object under examination to be viewable through the camera of the smartphone.

The outcomes of calibrating an ocular micrometer against an objective micrometer, while employing an objective lens set at a specific magnification (e.g., 4X), are subsequently utilized to determine the length of an object observed through an objective lens at the same magnification (e.g., 4X). An instance of quantifying the dimensions of an item having an elongated cross-section, namely a leaf, is demonstrated through the utilization of 4x magnification. This process yields a leaf thickness measurement of 500 um. The data presented in Figure 9 was derived from ocular lines measuring 20 x 25 um.

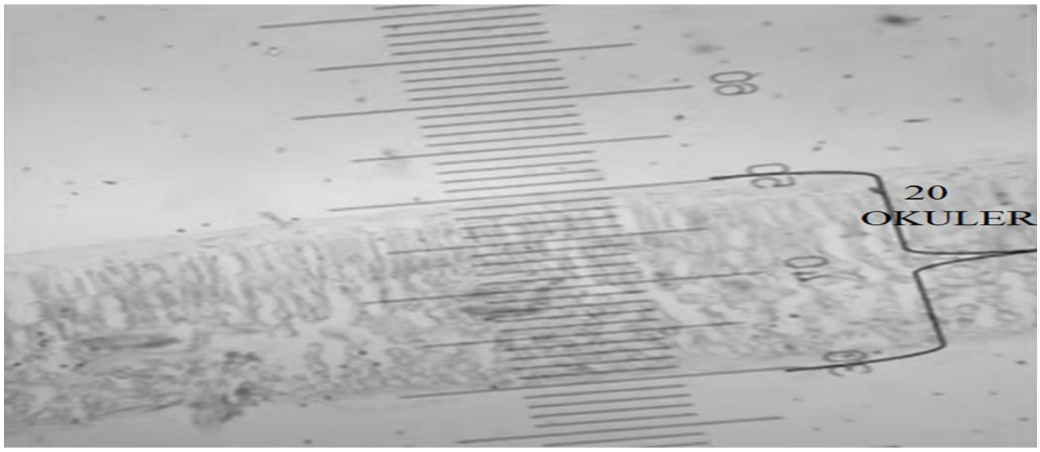

Fig 9 Measurement of Leaf Thickness in Observations using a 4X Objective Lens

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The aforementioned findings correspond to the measurements obtained through the utilization of the objective lens with a magnification factor of 10X. The outcome is 500 micrometers, as seen in Figure 10.

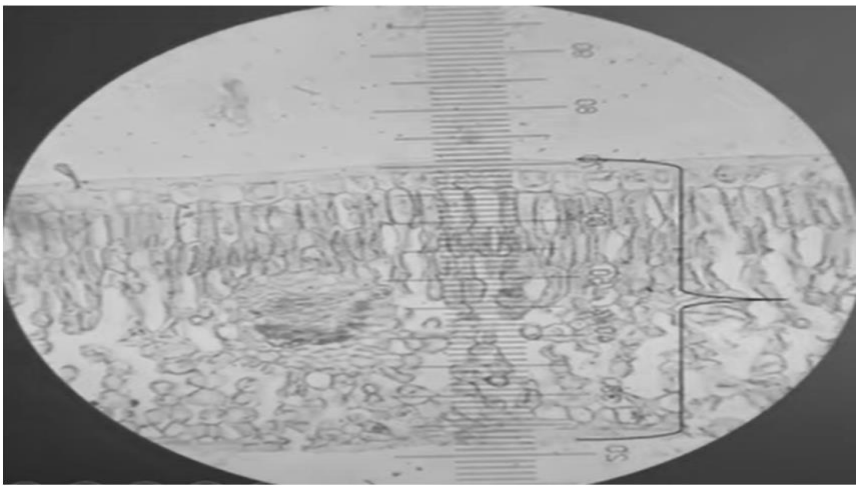

Fig 10 Measurement of Leaf Thickness in Observations using a 10X Objective Lens.

The utilization of a digital camera, in conjunction with a mobile phone, for the purpose of quantifying and capturing images on a microscope is feasible. The implementation of this technology involves the installation of a camera that is linked to an adaptor, which is selected based on its size. For this particular instance, a 1/2 threaded and perforated pipe junction with a size of 1/3 was employed, which underwent alteration by the application of heat to the lining within the pipe orifice using solder. The process of heating is conducted until the desired dimensions of the aperture are achieved, enabling the insertion of the ocular lens. Moreover, the methodology for calibrating and using the outcomes of scale calibration to quantify items in a microscope bears resemblance to the procedure of operating a telephone.

Measurements can be conducted utilizing the ImageJ software subsequent to capturing an image of the item using a cell phone or digital camera. The utilization of this software entails an initial step of program installation. After installation, the software is launched and utilized. The application allows for the opening of a file containing a photo or image that is to be measured. Adjustments or modifications to the set or scale settings can be performed using the menu options that are now accessible. The scale remains a widely employed reference point for establishing measuring standards. The process of measuring an object involves the extension of a line from an initial point to the terminal point that is to be quantified on the object.

IV. CONCLUSION

The training activities conducted at the Cenderawasih Polytechnic Campus successfully met the objectives in enhancing the knowledge, attitudes, and competencies of the faculty members in utilizing biophotomicrograph methods for instructional, learning, and research purposes. Furthermore, the outcomes of the training activities led to the development of a modified photomicrograph design, which was effectively employed for international standard measurements.

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