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Digitalization of Microscopic Cell Analysis Using ImageJ and Micrometers as an Innovation in Science Experiments in the Era of Industrial Revolution 4.0

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ABSTRACT

Cell measurement and analysis are fundamental skills in biology and science education microscopic practicums. The purpose of this study is to compare the accuracy, consistency, and processing time of onion cell measurements using an ocular micrometer as a traditional method and ImageJ software as a digital method. The digitalization of microscopic cell analysis as an innovation in science education supports the 4.0 Industrial Revolution. The research employed a comparative-experimental design. This study's sample consisted of 270 undergraduate students from a university with basic experience using micrometers and microscopes, ensuring that the participants' technical abilities were relatively homogeneous. The data analysis for this study focuses on comparing cell size measurements and processing time between the two methods. The results show a non-significant difference in cell size measurements and significant differences in processing time between the traditional and digital methods, corresponding to a large effect size and a reduction in processing time for the digital method. The digital method is more efficient. The results of this study suggest that the digitalization of microscopic cell analysis not only empowers technology integration in science education but also supports the development of students' digital literacy and analytical skills.

ARTICLE INFORMATION

Keywords:
cell measurement; digital microscopy; imagej; industry 4.0 education; science practicum

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INTRODUCTION

Cell measurement is a fundamental skill in microscopy-based laboratory work and science education. This skill is not only about the technical ability to operate a microscope but also about understanding the relationship between morphological structures and physiological functions in organisms (Herawati et al., 2024). Cell measurements can be used to analyze variations in cell size across tissues (Bourn et al., 2025), compare cells under normal and abnormal conditions, and evaluate morphological changes associated with pathological conditions (Hu et al., 2021; Stenkula & Erlanson-Albertsson, 2018). Cell measurement activities also play an important role in developing scientific thinking skills, such as observation, data analysis, and drawing conclusions based on microscopic images (Harefa et al., 2025; Pratama, R. et al., 2025). Therefore, the levels of accuracy and efficiency in cell measurement are determining factors in the quality of microscopic analysis results in both learning and research contexts.

Microscopic laboratory activities still often face various obstacles that affect students' learning experiences. Observation and measurement of cells are carried out by participants alternately due to the limited number of microscopes, so that not all participants have the same learning opportunities (Kurniawan, 2025; Makin et al., 2023). In addition, the relatively short practicum duration meant that manual measurements with an ocular micrometer were often carried out hastily, resulting in inaccurate data (Djunaid & Bannang, 2025; R. Pratama & Cahaya, 2025). The situation is further complicated by variations in participants' technical abilities in performing calibrations and observations, which can introduce subjective bias and lead to inconsistencies in data between observers (Britten-Jones et al., 2022; Out et al., 2025). This condition indicates that cell measurement skills have not yet been fully optimized during the learning process.

Conventionally, cell measurements can be performed using a light microscope equipped with an ocular micrometer. This method has been widely used and is regarded as the standard approach in basic learning and research. However, several limitations need to be considered (Yoda, 2022). The calibration and measurement processes require high precision and are relatively time-consuming (Culley et al., 2024). In addition, manual measurements are highly influenced by the observer's subjectivity, skills, and experience, potentially resulting in inter-observer bias (Britten-Jones et al., 2022; Out et al., 2025). Inconsistency can reduce the reliability of practical results. This condition poses a challenge, especially in practical activities involving a large number of participants, limited time, and many objects to be measured.

Meanwhile, technological developments in the era of the Industrial Revolution 4.0 have driven the transformation of science learning towards digitalization (Anindita et al., 2024; Pratama, S. et al., 2025). The use of computer-based image processing software offers a potential solution to improve the efficiency and accuracy of plant cell size analysis in microscopic practicums (Feng et al., 2023; Jayakody et al., 2017; Umam et al., 2023). An image is a two-dimensional representation of objects from the visual world, spanning disciplines such as art, human vision, astronomy, and engineering (Jumadi et al., 2021). Image processing refers to any form of signal processing in which the input is an image, such as a photograph or video frame, and the output can be either an enhanced image or a set of characteristics or parameters derived from it (Andrian et al., 2022).

Several studies have shown that the use of digital technology in microscopic visualization can improve image display quality and documentation of observation results (Amien & Basari, 2023; Dewantara et al., 2022; Gabdulinova & Kovrova, 2021). However, most of these studies have focused on demonstrating the applicability of digital tools such as ImageJ in isolation, rather than directly comparing their accuracy and processing time efficiency with the conventional ocular micrometer method within the same sample. Consequently, empirical evidence on the extent of accuracy and time gains achieved by switching to digital measurement in an educational setting remains limited, even though such evidence is crucial for designing technology-based practical work and learning activities. This study addresses this gap by directly comparing the two methods on the same specimens and the same group of students, offering empirical evidence to inform the design of technology-based practicum modules. Digital methods not only offer faster, more accurate analysis but also support innovation in science education (Chintalapati et al., 2017).

One of the image processing tools commonly used in microscopic practices is ImageJ (Mohamed et al., 2019). ImageJ enables semi-automatic processing of microscopic images through standardized calibration, allowing measurements to be performed with higher precision and reducing processing time compared to manual methods (Negi & Singh, 2023; Ramm et al., 2024). Measurements obtained using ImageJ can be documented as digital images that can be reanalyzed, tested for consistency, and provide more detailed results, making it highly valuable for applications in cellular biology education and research (Li et al., 2007; Satriorini et al., 2022). The application of ImageJ enhances laboratory

digital literacy, scientific analysis skills, and user independence in processing microscopic data. Based on this description, this study is important to compare the effectiveness of cell measurement using an ocular micrometer as a traditional method and ImageJ software as a digital method in terms of measurement accuracy and time efficiency. This study also evaluates the potential integration of digital technology into science laboratory work. The findings are expected to offer more efficient and accurate laboratory alternatives that align with 21st-century competency requirements, as well as provide a reference for developing technology-based laboratory modules and instructional designs in the digital era.

METHOD

This study used a quantitative, comparative experimental design to evaluate two methods for measuring red onion cells. The traditional method used an ocular micrometer, while the digital method used ImageJ for image analysis. Subject selection was conducted purposively, involving 270 science practicum students with basic experience in microscopic observation, enabling them to understand microscope procedures and perform measurements independently. The instruments used included a light microscope equipped with an ocular micrometer for manual measurement, red onion epidermal tissue specimens as the objects of observation, ImageJ software for digital measurement, and a stopwatch to record the time required for the measurement. Each measurement method was assessed based on cell size results and measurement duration. The overall research stages are presented in Figure 1.

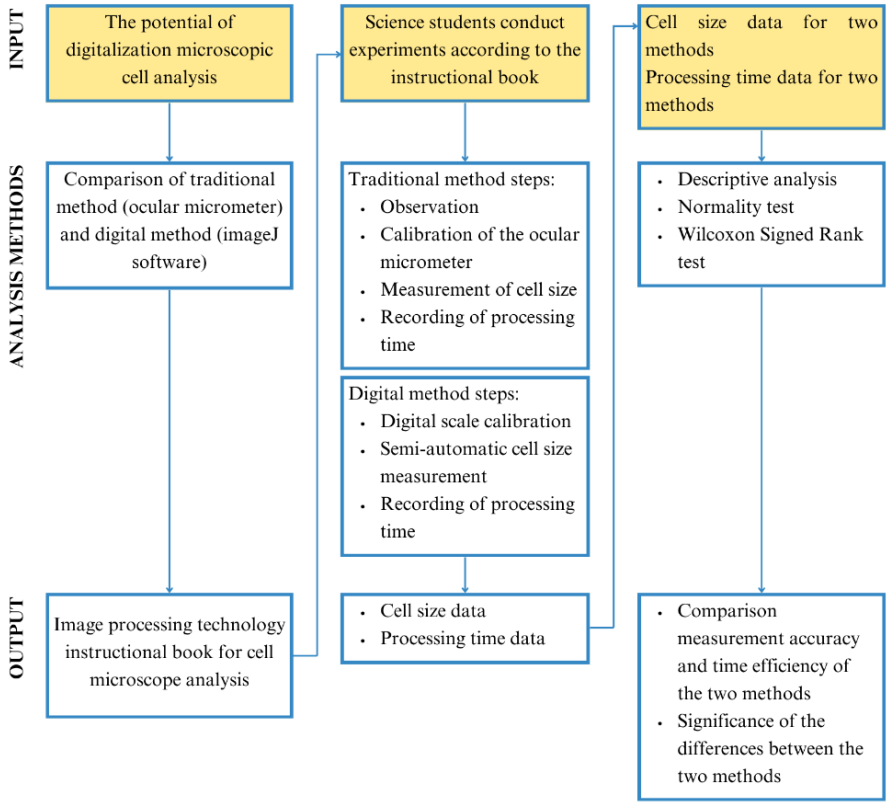
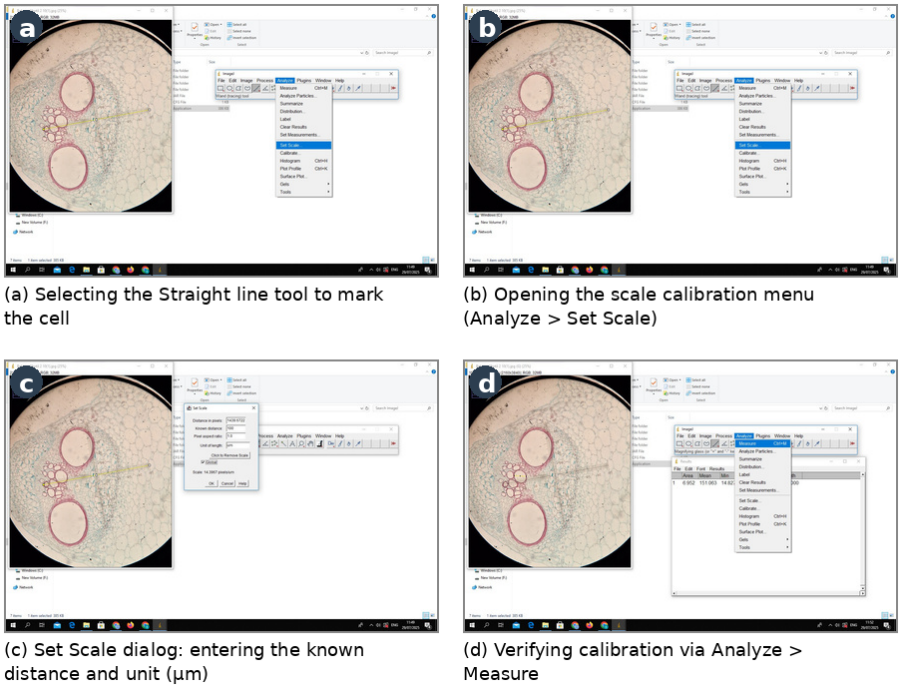


Figure 1. Research Stages

The experimental stage was carried out by students using two methods, as outlined in the instructional book. In the first stage, students observed onion epidermis preparations under a microscope at 4x magnification, calibrated the ocular micrometer against an objective micrometer,

measured cell size for 3 cells per specimen to obtain an average value, and recorded the time required for the process. In the second stage, students used the same microscopic images for digital analysis. Each image was first calibrated in ImageJ using the Set Scale function, with a known reference distance (100 µm) entered and the measurement unit set to micrometers, ensuring that all subsequent measurements were expressed in calibrated units rather than raw pixels. Cell boundaries were then marked using the Straight-line tool, and cell size was measured using the Analyze > Measure function. This measurement was repeated for three cells per specimen, with each region of interest stored in the ROI Manager to ensure traceability and consistency of the measured areas. The time required to complete calibration and measurement was recorded using a stopwatch for both methods. This standardized calibration procedure for both the ocular micrometer and ImageJ supported the validity of the measurements, as both methods were referenced to the same known-distance standard prior to data collection. The procedures are displayed in Figures 2 and 3.



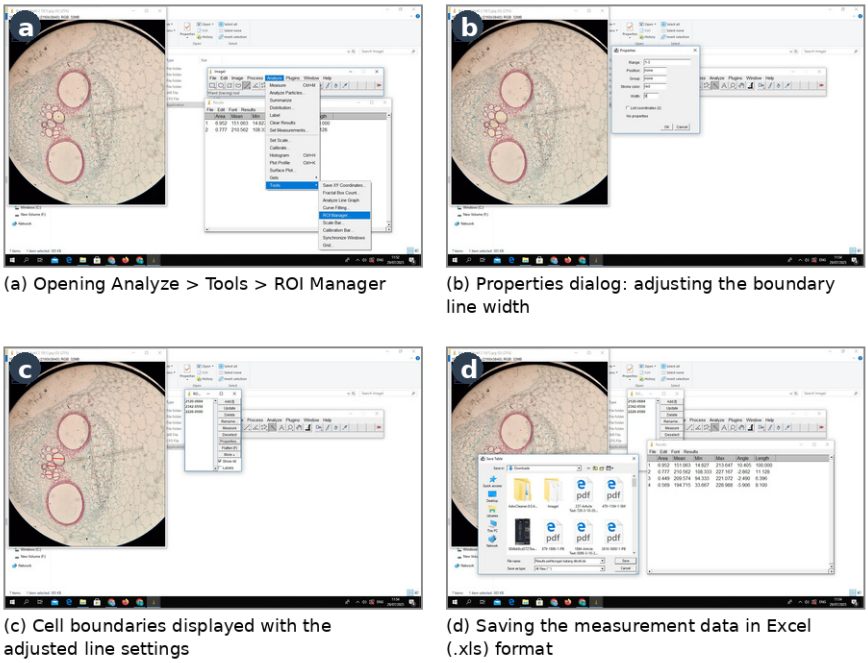
(a) Selecting the Straight line tool to mark the cell

(b) Opening the scale calibration menu (Analyze > Set Scale)

(c) Set Scale dialog: entering the known distance and unit (µm)

(d) Verifying calibration via Analyze > Measure

Figure 2. ImageJ calibration and measurement procedure



(a) Opening Analyze > Tools > ROI Manager

(b) Properties dialog: adjusting the boundary line width

(c) Cell boundaries displayed with the adjusted line settings

(d) Saving the measurement data in Excel (.xls) format

Figure 3. Repeated measurement and data storage in ImageJ

Data analysis was performed quantitatively and descriptively. All measurement results and processing times were tabulated and processed using SPSS version 25 to calculate the mean, standard deviation, and data distribution. Prior to hypothesis testing, a normality test was conducted for each group using the Shapiro-Wilk test, given its higher sensitivity for moderate sample sizes. The results indicated that both cell size and processing time data were not normally distributed across all four groups ($p < 0.05$ for cell size; $p < 0.001$ for processing time). Based on this result, the non-parametric Wilcoxon Signed-Rank test was selected to identify differences between the two paired, non-normally distributed data groups, rather than a paired-samples t-test.

RESULTS AND DISCUSSION

This study compares cell size measurements and processing time between the traditional method using an ocular micrometer and the digital method using ImageJ. Based on 270 measurements, the average red onion cell size measured using the traditional method was 99.53 µm. Meanwhile, ImageJ measurements yielded an average size of 99.62 µm. The difference between the two methods was only 0.09 µm, indicating that the digital method achieved accuracy equivalent to that of the micrometer method. The data distributions for both methods were also similar, as indicated by standard deviation values of 49.58 for the traditional method and 49.52 for the digital method. The data are shown in Table 1, while Figure 2 compares the distributions of shallot cell sizes between the traditional and digital methods using boxplots.

Table 1. Comparative Descriptive Statistics of Cell Size Measurements between Two Methods

Cell Measurement	N	Mean (µm)	Std. Deviation	Minimum	Maximum
Traditional Method	270	99,53	49,58	16	262.00
Digital Method	270	99,62	49,52	15,77	261.30

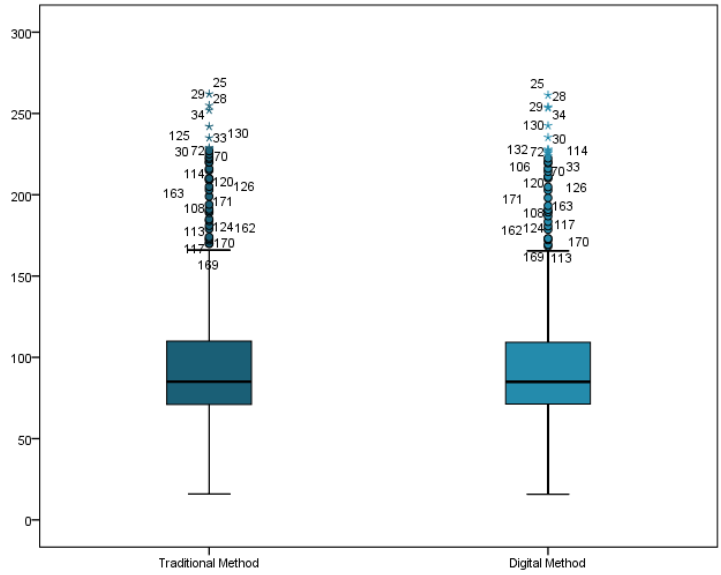


Figure 4. Cell size of two methods

The medians of both methods in Figure 4 are in the same range, and the quartile positions (Q1–Q3) are close to each other. This indicates that the data distribution of both methods is relatively similar. The almost identical interquartile ranges (IQRs) also indicate that the data variation in both methods is not significantly different. The number of outliers appearing in both boxplots is also relatively balanced, indicating that both traditional and digital methods have equivalent extreme distribution patterns. The similarity of these boxplot patterns reinforces the statistical test results that the digital method achieves measurement accuracy comparable to that of manual measurements using an ocular micrometer. The average cell size per group is shown in Figure 5.

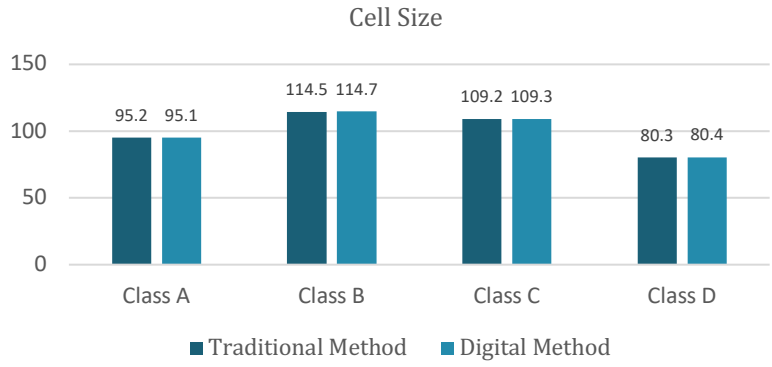


Figure 5. Average cell sizes for each class

Figure 5 indicates that both methods produce stable values across all practitioner groups. The average differences between groups are very small and are within a similar range, both in the traditional and digital methods. This pattern shows that the measurement results of both the micrometer and ImageJ methods are consistent between practitioners, with no group deviating significantly from the others. The consistency in the traditional method is due to the subjects being selected purposively, namely students who already have basic experience using micrometers and microscopes, so that the technical abilities between practitioners are relatively uniform. In addition, all participants measured the same specimen, namely a single slice of onion cell with a clear structure and easily identifiable cell boundaries, thereby reducing differences in perception between

individuals. The measurement process was also carried out over a sufficient period of time so that participants were not rushed, which could increase the risk of error.

Meanwhile, digital methods are, in principle, capable of achieving high consistency by using image segmentation algorithms to detect cell boundaries. The consistency of this data is in line with previous studies which confirm that the use of digital image processing software, such as ImageJ, can improve accuracy while reducing observer subjectivity compared to manual methods (Fadjeri et al., 2023; Rahimi et al., 2023). Kadam et al. (2024) compare leaf area measurements for 10 pomegranate genotypes using manual and digital methods in ImageJ. The leaves were photographed, then analyzed in ImageJ to automatically separate leaf objects and calculate their areas. The results showed variations in leaf area between genotypes and a significant correlation between the two methods, thus confirming ImageJ as a fast, accurate, and non-destructive method.

Consistency in cell measurements using digital methods is important, as manual methods often depend heavily on the accuracy of the individual observer (Cizkova et al., 2021). For example, when determining cell boundaries, observers may interpret cell edges differently, resulting in high inter-observer variation. In contrast, a computer-based approach uses image segmentation algorithms to detect cell boundaries, resulting in more objective and consistent results (Sakdiah, 2024). ImageJ provides various types of measurements such as areas, diameters, perimeters, angles, circularity, coordinates in 2D and 3D, intensities, calibrated pixel values, and is capable of generating a variety of statistical analyses, enabling the detection of finer morphological details compared to manual methods (Panda et al., 2024; Prodanov & Verstreken, 2012). Therefore, the accuracy and consistency of ImageJ results can be considered a valid alternative to practical work in microscopy. The results of the Wilcoxon Signed-Rank test are presented in Table 2.

Table 2. Wilcoxon test results

Data	Z	P Value
Cell Measurement	1,105[a]	0,269
Processing Time	14,140 [b]	0,000

a. Cell size traditional method > cell size digital method
b. Processing time traditional method > processing time digital method

The results of the Wilcoxon Signed-Rank test in Table 2 were used to examine differences in cell size measurements and processing time between the traditional and digital methods. The analysis yielded a Z value of 1.105 with a p-value of 0.269 ($p > 0.05$), indicating that there is no statistically significant difference between the two methods for measuring cell size. This finding is consistent with the descriptive statistics and boxplots, which show comparable means, medians, and variability ranges for both methods. Although individually there are some conditions where the cell size measured using ImageJ is slightly larger or smaller than that measured using a micrometer, these differences are not systematic or consistent enough to produce a statistically significant effect. This suggests that the digital method provides measurement results comparable to those obtained through conventional manual techniques.

An effect size analysis was conducted using the formula $r = Z/\sqrt{N}$ to evaluate the magnitude of the difference further. The result ($r \approx 0.07$) indicates a very small effect size based on Cohen's (1988) criteria. This negligible effect suggests that the difference between the two methods is not only statistically non-significant but also practically insignificant. Therefore, in the context of laboratory and educational applications, the digital method can be considered to produce measurement outcomes that are functionally equivalent to those obtained using a micrometer. In contrast to the cell size results, the comparison of processing time revealed a statistically significant difference between

the two methods. The Wilcoxon Signed-Rank test yielded a Z value of 14.140 ($p < 0.001$), indicating that participants required significantly more time using the traditional method than the digital method. The direction of the difference consistently indicates that the digital method enables faster measurement. The average processing time using an ocular micrometer was 15.7 seconds (SD = 11.3), whereas measurements using ImageJ required only 6.9 seconds on average (SD = 4.2), as shown in Table 3.

Table 3. Comparative Descriptive Statistics of Processing Time Measurements between Two Methods

Processing Time	N	Mean (minutes)	Std. Deviation
Traditional Method	270	15.7455	11.37239
Digital Method	270	6.9502	4.20995

An effect size analysis was conducted using the formula $r = Z/\sqrt{N}$ to assess the magnitude of this difference. The resulting value ($r \approx 0.86$) indicates a large effect size based on Cohen's (1988) criteria. This suggests that the observed difference is not only statistically significant but also substantial in practical terms. From a practical perspective, the digital method reduced the average processing time by approximately 56% compared to the traditional approach. In laboratory and educational settings, particularly those involving large groups of students or repeated measurements, this level of efficiency can meaningfully optimize time allocation, allowing more opportunities for data analysis, interpretation, and conceptual discussion rather than time-consuming manual procedures. The average processing times for the two methods are presented in Figure 6.

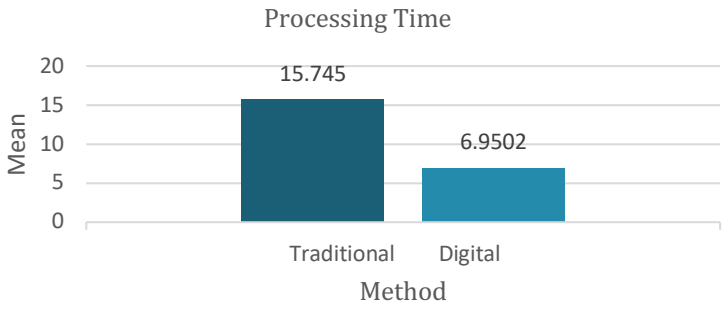


Figure 6. The average processing time of the two methods

The analysis shows that although manual measurements with a micrometer can still be performed, the process is slower because observers must repeatedly calibrate, adjust the microscope focus, and record results manually. In studies with large sample sizes, this workload becomes even heavier. In contrast, using ImageJ enables automated image processing. Once the image is obtained, cell measurements can be performed more quickly using the set scale and measurement tools features. This process can be repeated for many images in much less time than it takes for manual measurements.

ImageJ allows users to automatically import pixel size information in microns from image metadata or manually set the measurement scale when metadata is unavailable. This feature ensures that every morphological measurement is recorded in calibrated units, thereby improving the accuracy and consistency of results. In addition, the automated calibration process speeds up the analysis stage and significantly improves time efficiency compared to manual scale determination in traditional methods (Schroeder et al., 2021). ImageJ software is efficient, time-saving, and easy to use for processing large numbers of samples (Umam et al., 2023). Manual methods require weeks for plant biology research that involves measuring thousands of cells (Schroeder et al., 2021). However, with

image processing software, the same work can be completed more quickly. ImageJ-based technology can be used to process large-scale microscopic image data (Jadzic et al., 2021).

This time efficiency generated by the digital method is very relevant for practical work in large classes. In classes with a large number of students, time is a major constraint, especially when students must calibrate micrometers one by one and record the results manually. ImageJ reduces this technical burden by enabling images to be analyzed more quickly and reprocessed without re-observation under a microscope. This allows practical activities to continue optimally even when observing many samples with limited time. Another advantage of the digital method is its ability to store, reprocess, and document images (Mendes et al., 2025). Images generated by ImageJ can be reanalyzed to ensure measurement accuracy and serve as visual documentation unavailable in traditional methods. This process provides in-depth learning by allowing students to see the relationship between microscopic objects, such as cells, and quantitative data from cell-size measurements simultaneously (Nurjannah et al., 2024). In university science education, the use of ImageJ enriches students' laboratory learning. Students not only practice microscopic skills but also develop digital literacy, data analysis, and scientific image interpretation skills.

Despite these advantages, the digital method also has several limitations that should be acknowledged. First, ImageJ requires an initial learning curve, as users need to become familiar with its calibration and measurement tools before they can operate the software independently and efficiently. Second, because calibration in ImageJ is performed manually through the Set Scale function, an incorrect input of the known reference distance can introduce systematic measurement error that is not immediately obvious to the user. Third, the accuracy of digital segmentation and boundary marking depends on the quality of the microscopic image itself; poor lighting or an out-of-focus capture can make cell boundaries harder to identify precisely, regardless of the software's capability. These limitations indicate that the benefits of digital measurement depend on adequate image quality and basic user training, rather than being automatic advantages of the software itself.

From an institutional perspective, however, these efficiency gains also depend on the availability of computers or laptops for every student or group, technical support, and sufficient orientation time for software use; without such infrastructure, the time savings observed in this study may not be readily reproduced in institutions with limited digital resources. In addition, because ImageJ performs boundary detection and unit conversion automatically once calibrated, students may operate the software procedurally without fully engaging with the underlying measurement principles that manual micrometer use makes explicit, representing a trade-off between efficiency and conceptual engagement that should be considered when designing digital-based practicum modules. These considerations do not undermine the technical validity of ImageJ, which has been independently confirmed in prior validation studies comparing ImageJ-based quantification against reference standards (Helmy & Abdel Azim, 2012; Schindelin et al., 2015), but they indicate that its educational benefits are conditional on adequate infrastructure and pedagogical design rather than automatic.

Similar time–effort trade-offs have also been observed in other ImageJ-based applications; for instance, Elliott et al. (2022) found that manual ImageJ-based segmentation of plant disease lesions, while accurate, required an individually created mask for every image, making it substantially more time-consuming than a machine-learning-based alternative, particularly for larger datasets. For a practicum-scale application such as the present study, involving 270 measurements, the manual calibration and boundary-marking steps in ImageJ remain manageable; however, institutions seeking to scale digital cell measurement to larger cohorts or repeated semesters may benefit from exploring semi-automated or machine-learning-assisted segmentation approaches to further reduce processing time and observer input.

The integration of image processing technology is also in line with the demands of the digital era in science education. Science curricula in higher education increasingly emphasize digital literacy and data analysis skills. The use of ImageJ is not only relevant for research, but also strategic for higher education. Based on the findings of this study, ImageJ has great potential for incorporation into a digital or blended learning practicum module. Microscopic images can be prepared in advance and shared with students for independent analysis, both in and out of the classroom. Practicums are no longer limited to the availability of physical microscopes. This approach aligns with the need for flexible, efficient, and adaptive learning in the era of digitalization in higher education. This finding is consistent with recent reviews indicating that digital imaging tools are increasingly reshaping laboratory instruction across science disciplines, extending meaningful quantitative analysis to students in remote or resource-limited settings and promoting greater learner autonomy and inquiry-based engagement (García Ramos et al., 2025).

In the context of cell measurement practicum specifically, the time saved through digital analysis can be redirected toward higher-order learning activities, such as interpreting the biological significance of cell size variation, discussing sources of measurement error, or comparing results across specimen types, rather than being consumed entirely by repetitive manual calibration. Embedding ImageJ within a structured digital literacy framework, one that addresses not only software operation but also data interpretation and critical evaluation of automated output, would allow institutions to convert the time efficiency demonstrated in this study into genuine gains in students' analytical and digital competencies rather than a simple acceleration of the existing practicum workflow.

A similar pattern of trade-offs between digital and conventional methods has been reported outside plant and cell biology education. In a comparison of light microscopy (LM) and virtual microscopy (VM) among second-year medical students, Başer & Büyük (2024)found that VM was associated with significantly higher examination performance, while LM received significantly higher satisfaction scores, with students citing the hands-on, tactile nature of physical slide examination and the closer one-to-one guidance available during LM sessions. This parallels the pattern observed in the present study, where the digital method offered a clear, measurable advantage (time efficiency) without necessarily surpassing the traditional method in every respect, and reinforces the argument that digital tools are best positioned as a complement to, rather than a wholesale replacement for, hands-on microscopic practice. Taken together, these findings across different areas of science and medical education suggest that the practical value of digitalization lies primarily in efficiency and accessibility gains. At the same time, direct instrument-based practice continues to contribute uniquely to student engagement, motivation, and skill development that purely digital tools may not fully replicate.

In general, the results of this study indicate that the digitalization of microscopic cell analysis using ImageJ is an innovation in science laboratory practice that aligns with the demands of the Fourth Industrial Revolution. The accuracy of the digital method is comparable to that of traditional methods, improving processing efficiency, data consistency, and opportunities for more flexible, technology-based learning development. The digitalization of microscopic cell analysis is a strategic step in preparing students for an education and research increasingly oriented towards data and digital technology. This study is not without limitations. The comparison was conducted using a single type of specimen, namely red onion epidermal cells, so the findings may not fully generalize to other tissue types with more complex or less distinct cell boundaries. In addition, all participants had prior basic experience with microscopes and micrometers; the time and accuracy advantages of the digital method observed here may differ for novice users who are unfamiliar with either measurement technique. Future studies involving a wider range of specimens and participants with varying levels

of prior experience would help clarify the extent to which these findings can be generalized. Processing time in this study was recorded manually using a stopwatch; although this procedure was applied consistently across both methods, it may introduce minor timing variability. The study also did not assess whether the efficiency gains from digital measurement translate into improved learning outcomes, such as conceptual understanding of cell structure or long-term retention of measurement skills, an important direction for future research combining performance data with learning-outcome assessments.

CONCLUSION

The results of this study show that cell size measurements obtained with ImageJ are statistically and practically comparable to those obtained with the conventional ocular micrometer, with a negligible effect size ($r \approx 0.07$); the digital method does not improve measurement precision beyond the manual method. Instead, the clearest and most practically significant advantage of ImageJ lies in its processing time efficiency, evidenced by a large effect size ($r \approx 0.86$) and an average time reduction of approximately 56% compared to the manual method. This time efficiency, rather than superior accuracy, is what makes ImageJ a strategic tool for practical implementation in science practicum, particularly in large classes with limited time and equipment. This digital-based practicum aligns with the demands of modern science education in the 4.0 era, which emphasizes the integration of ICT into laboratory activities. To optimize its implementation, training for teachers and students on the use of ImageJ is needed, along with the development of digital practicum modules, adequate computing infrastructure, and instructional strategies that direct the time saved toward higher-order learning activities. Further research is also recommended to evaluate the impact of using this digital method on students' critical thinking skills, science literacy, and long-term retention of measurement competencies.

DECLARATION OF GENERATIVE AI

The authors declare that no generative AI or AI-assisted technologies were used in the writing process of this manuscript.

CONFLICTING INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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