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Original Article

Application and Accuracy of the Free Image Analysis Software for Tree-ring Measurement Compared with the Standard Tree-ring Measuring System

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ABSTRACT

Accurate ring width data is an important precursor for tree-ring analysis. This study aimed to apply image analysis software, namely ImageJ, to measure the annual ring widths of *Pinus kesiya*, as a case study, and compare its accuracy with the standard measurement derived from the Velmex measuring system. The accuracy of measurement was compared by using the Verify for Windows software and the paired sample t-test. It was found that ring width data from these measuring techniques were significantly correlated (r = 0.99; p = 0.000) and the measurements in each increment core were not significantly different. The application of climate-growth responses has shown the similarity of the relationship between ring width data derived from the two techniques and climatic data. Therefore, to increase the capacity and opportunity in tree-ring study, the image analysis using ImageJ software can be applied and its accuracy is not significantly different from that of a standard instrument under the conditions of proper sample preparation for image scanning and the correct cross-dating.

Keywords: Dendrochronology, ImageJ, Image analysis, Tree-ring analysis, Tree-ring measurement

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INTRODUCTION

One of the important processes in the tree-ring analysis, besides the cross-matching and cross-dating process, is the careful measurement of the annual ring widths with a recognized and high-precision instrument. The most well-known and widely used instruments for direct annual ring measurement are Velmex Tree Ring Measurement System (TA) and LinTab™ Measuring Tool (LTMT) (Velmex Inc., 2016; Rinn Tech., 2010), while the application of image analysis by using the specific software such as WinDENDRO (Guay, 2014; Regent Instruments Inc., 2017) is known and widely used as well. On the basis of correct cross-dating, both TA and LTMT were developed to be able to directly measure the annual ring width at a resolution of 1 micron (0.001 mm).

The TA system consists of a sliding linear stage integrated with a one-micron resolution linear encoder and the encoder readout connected to the linear encoder and a computer for measurement recording. MeasureJ2X (VoorTech Consulting, 2000), a tree-ring measuring program, is available to support the TA system for illustrating, transferring, and recording annual ring width data into the computer, while the registration key for the licensed software is separately purchased. A stereo microscope with boom standing and light source are other supporting parts of the TA system additionally installed to clarify and magnify annual ring structures and boundaries under a microscope. Ring width data measured by using the TA system associated with MeasureJ2X software can be directly analyzed by using free, famed, and acceptable tree-ring analysis software such as DPL, COFECHA, and ARSTAN (Holmes, 1983a; 1983b; Cook *et al*., 2017).

Slightly different from the TA system, the LTMT has a linear tree-ring measuring stage with a base connected to a microscope stand and uses the TSAP-Win™ software (Rinn Tech., 2010) for annual ring width measurements and analysis without additional tree-ring analysis software such as DPL, COFECHA, and ARSTAN (Holmes, 1983a; 1983b; Cook *et al*., 2017). However, ring width data derived from LTMT can also be analyzed by using these tree-ring analysis software. Another tree-ring measuring system, called WinDENDRO, can measure tree-ring width from stem disks, wood cores, and X-ray films using an image analysis system. The specific scanner with a maximum scanning area of 30x43 cm comes with a system that can generate high-resolution tree-ring images for ring width measurement and analysis by using WinDENDRO software.

These three types of tree-ring measuring systems with licensed software are quite costly and it is difficult to provide enough of them for research and educational needs. In addition, due to the situation of the COVID-19 pandemic, the government and university issued regulations to prevent the spread of the disease by allowing online learning, whereby students and researchers are unable to come into the laboratory for training and using tree-ring measuring systems.

To eliminate the limitation of insufficient instruments and licensed software for research and educational purposes, this study aimed to apply free software for image analysis, namely ImageJ software (Ferreira and Rasband, 2012), to measure the annual ring widths and compare its accuracy with the standard measuring system. The application of the image analysis technique can provide the opportunity to access the precise tree-ring analysis for those who do not have the standard measuring system. Students and interested persons can practice by themselves through personal computers in situations of a disease pandemic.

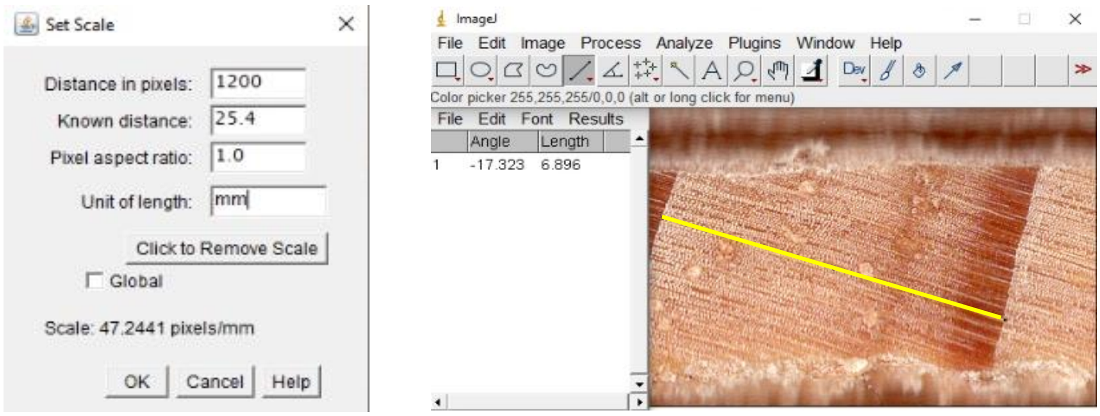
MATERIALS AND METHODS

Tree-ring samples

Twenty cross-dated tree ring samples of Khasi pine (*Pinus kesiya* Royle ex Gordon) obtained from the Laboratory of Tropical Dendrochronology (LTD) were selected for this study. These samples were randomly collected from the healthy mature standing trees using an increment borer at the Wat Chan Pine Forest under the responsibility of the Forest Industry Organization (FIO) in Chiang Mai Province, north of Thailand. All samples were prepared following the standard method of dendrochronology (Stokes and Smiley, 1996), including sample mounting on a supporting wood, polishing by using several grades of sandpaper until the rings are clearly visible, and cross-matching to specify the growing year of each annual ring, and kept at room temperature in the LTD sample room.

Tree-ring measurement

The measurement of annual ring widths were divided into 2 parts. The first part was a standard ring width measurement which was done using the TA system. Ring width data were then transferred and recorded in a microcomputer using the MeasureJ2X software (VoorTech Consulting, 2000). The second part was ring width measurement by using the image analysis which was started by increment core scanning with a high resolution of 1200-2400 dots per inch (dpi). One of the beware caution of the sample core scanning is the length fit between the sample cores and scanning area of a regular scanner which must not be longer than 30 cm. However, the scanning areas of specific scanners for tree-ring analysis are available up to 43 cm (Regent Instruments Inc., 2017). In this case, the 1200 dpi resolution was selected because the images of annual rings were clear enough for measurement. The image files were then saved in Tag Image File Format (TIFF). The ImageJ software was applied for image analysis of ring width measurement. Before measurement, the image scale was set at the image resolution of 1200 dpi equal to 25.4 mm or 25400 µm. Image scale setting and ring width measurement are shown in Figure 1.



(a) (b)

Figure 1 Image scale setting (a) and ring width measurement (b) using the ImageJ software.

Testing the validity of the annual ring dataset

Ring width data measured using the standard instrument was compared based on the similarity of growth fluctuation using the COFECHA software (Holmes, 1983b). The trends of ring width variations from all sample cores will be consistent if the specified growing year is corrected. Ring width measurement file in Tucson format derived from the MeasureJ2X software was directly applied to testing the validity of the dataset using COFECHA. The text file or spreadsheet derived from the ImageJ software was first re-formatted to Tucson format using the FMT, one of the options under DPL software (Holmes, 1983a), or in some other way before running the COFECHA.

Tree-ring index construction

The acceptable dataset of ring widths approved by the COFECHA in both the TA system and image analysis measurements were then used for tree-ring index construction. The tree-ring index is the representative of ring width dataset detrended and age effects and other endogenous stand disturbances removed (Fritts, 1976). In this case, ARSTAN software (Cook et al., 2017) was applied for tree-ring index construction and all commands used for construction in both measurement systems must be similar. Therefore, any differences in the indices should be due to the different ring width measurements. Fritts (1976) also suggested the quality of indices for climate studies in terms of high values of the mean inter-series correlation (Rbar), mean sensitivity (MS), and expressed population signal (EPS), and low value of autocorrelation (AC). The acceptable value of EPS is 0.85 and higher (Wigley *et al*., 1984).

The relationship of climate and tree growth

Multiple regression analysis was used to describe the climate-tree growth relationship. Monthly, seasonal, and annual climate data of rainfall and temperature derived from the Mae Hong Son meteorological station and the constructed tree-ring indices calculated using annual ring dataset from TA and image analysis systems were defined as independent and dependent variables, respectively. According to the dynamics of rainfall and temperature data, the seasons can be divided into wet and dry in May-October and November-April, respectively. The dry season can be subdivided into summer in March-April and winter in November-February as shown in the figure 2.

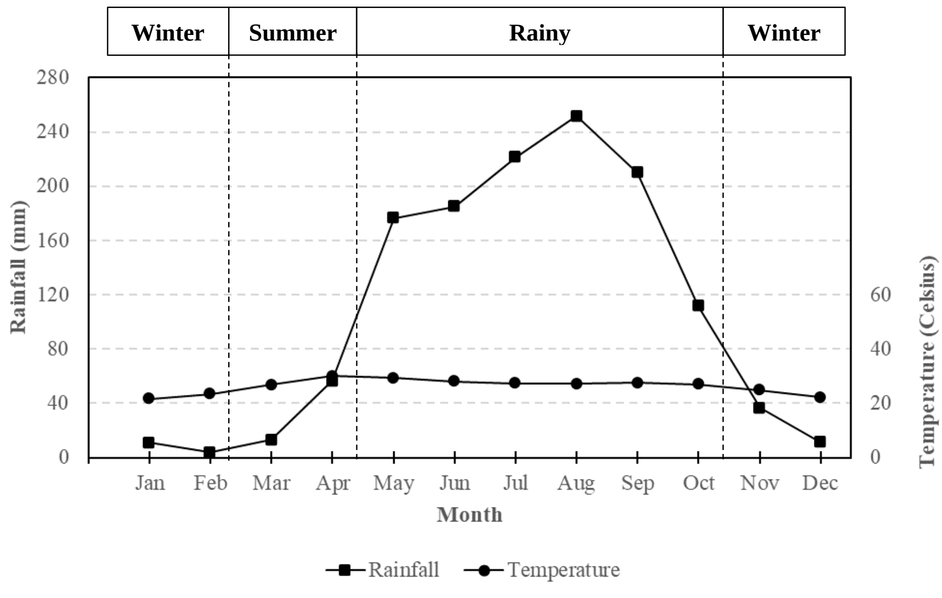


Figure 2 The average monthly rainfall and temperature data from the Mae Hong Son meteorological station from 1955 to 2015. It can be divided to 3 periods of summer, winter, and rainy seasons.

The comparison of ring width dataset derived from the standard instrument and the image analysis

In each increment core, the difference of measurement in each annual ring was calculated and the percentage difference from the measurements from the whole increment core was shown. Additionally, two ring width datasets derived from the standard instrument and the image analysis were compared using the software, namely, Verify for Windows (Lawrence and Grissino-Mayer, 2001), to check the accuracy of these two measurement techniques. From the software, Verify for Windows compared two ring width data from the same increment core and illustrated the result in four parts of comparative statistics, linear regression, verification, and outlier detection. The measurement was defined as a significant difference when the average squared difference between the measurements was greater than the confidence level of 0.01. Paired sample t-test was also used to confirm the differences between the two ring width datasets derived from the two measurement techniques.

RESULTS AND DISCUSSION

The validity of the tree-ring measurement

The 20 increment cores were measured using the standard and the image analysis techniques, with a total of 803 annual rings. The validity of the tree-ring dataset was done by COFECHA software which outputs from both techniques showed a slight difference in statistical values (Table 1). Cross-matching done before ring width measurements resulted in the same number of dated series, total rings, total dated rings, and length of master series. However, the variation in ring width measurements using different techniques induced small differences in the series intercorrelation and the average mean sensitivity. The series intercorrelation and the average mean sensitivity illustrated the association among data sets and the relative differences from one ring with to the adjacent ring, respectively. Maxwell *et al.* (2011) compared the differences of ring width measured by using the two standard techniques of TA system and WinDENDRO and found the similarity of ring width measurements between techniques. Segments with possible problems showed intercorrelation values under the 99% confidence level indicating the anomalous growth patterns of each tree over a given period when compared to other pine trees in the same stand.

Tree-ring indices

The 70-year tree-ring indices for Khasi pine (*Pinus kesiya*) had been established from two groups of ring width data from different measuring techniques. The average ring width derived from TA system and ImageJ were 4.41 and 4.42 mm, respectively. The ARSTAN software with the first detrending in the negative exponential or linear regression in any slope and the second detrending in the hugershoff growth curve was applied to construct tree-ring indices in both datasets. The Standard (STD), Residual (RES), and Arstan (ARS) indices with the averages of raw ring width (RAW) were generated from each dataset as shown in Figure 3 and the quality of indices were shown in Table 2. Pine growth rate rapidly increased from the beginning of the age in the 1950s to the end of the 1990s for 50 years and gradually declined in the last 16 years until 2016 when the sample cores were collected. The average ring width of *P. kesiya* growing in this site was almost equal to another nearby site studied by Naumthong *et al*. (2021) in the Intakin site (0.43 mm), which is only 142 km in distance and 65 km in displacement away from this site, but the growth trends were different. Khasi pines growing in the Intakin site rapidly declined after the first 10 years to the current year illustrating the more sensitivity to environmental factors (Mean sensitivity

(MS) = 0.364) at the beginning of their age, while Khasi pine growth in Wat Chan Pine Forest increased for 50 years before declining in the last 16 years showing the more complacent ring pattern (MS = 0.272 to 0.298) and reflecting the growth of Khasi pines at the beginning of their ages was not limited by environmental factors.

Table 1 The summary of COFECHA output derived from the standard and the image analysis techniques.

	TA System	ImageJ
Number of dated series (core)	20	20
Length of master series (year)	70	70
Total rings in all series (ring)	803	803
Total dated rings checked (ring)	797	797
Series intercorrelation	0.462±0.131	0.465±0.130
Average mean sensitivity	0.317±0.099	0.318±0.100
Segments with possible problems (segment)	4	4

Table 2 Quality of indices from the different tree-ring measuring techniques.

Technique	Index	AC	MS	Rbar	EPS
TA System	RAW	0.492	0.293	0.155	0.791
	STD	0.242	0.272	0.179	0.833
	RES	-0.092	0.287	0.359	0.833
	ARS	-0.089	0.299	0.333	0.804
ImageJ	RAW	0.497	0.291	0.156	0.794
	STD	0.240	0.274	0.181	0.837
	RES	-0.087	0.288	0.361	0.837
	ARS	-0.094	0.298	0.337	0.808

The statistical values of indices from both measuring techniques were slightly different. The ARS indices of these measuring techniques had a highest value of the mean sensitivity (MS) indicated the highest value of the relative difference of the index from one year to the next as a result of environmental factors, while autocorrelation (AC) approaching zero indicated the correlation of the index from one year to the next as a result of growth trend removal. However, it was suggested that the number of sample cores should be increased to strengthen the Rbar value and cause the EPS value to rise to 0.85. Based on the index quality as shown in Table 2, the ARS indices from both techniques were then selected to study climate-growth relationship. It was consistent with the explanation that the ARS index intended to contain the strongest climatic signal possible (Cook and Holmes, 1986).

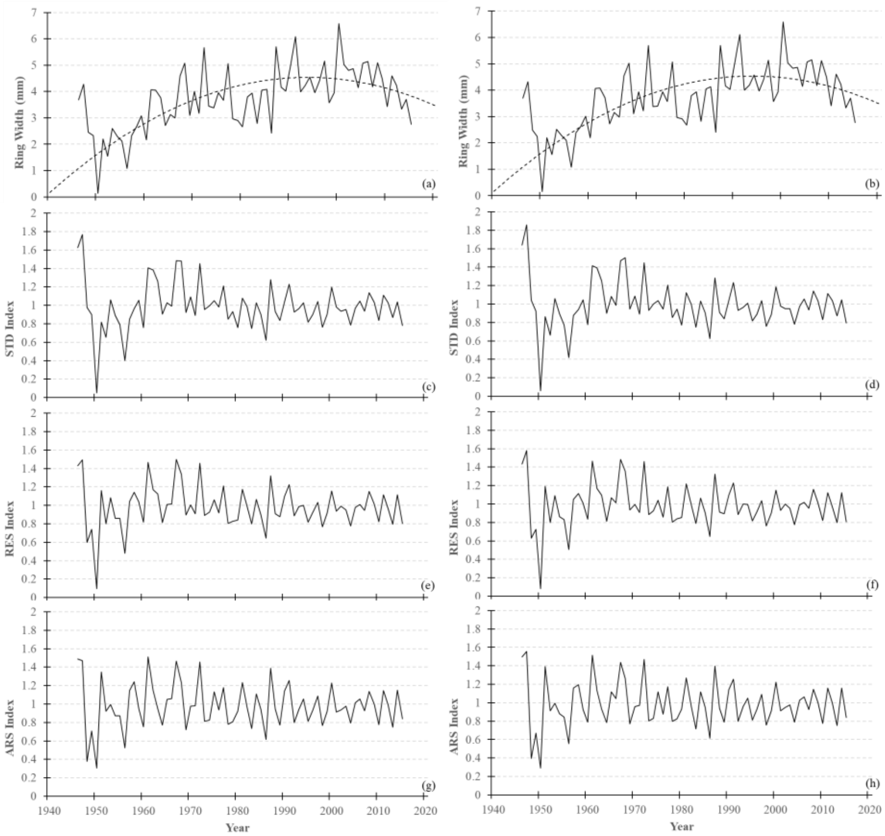


Figure 3 The chronologies of RAW (a, b), STD (c, d), RES (e, f), and ARS (g, h) derived from the standard and image analysis techniques for ring width measurement, respectively. Dashed lines in RAW chronologies (a, b) indicated pine growth trends.

Climate and tree growth relationship

The multiple regression analysis indicated monthly temperature in January (T_{Jan}) and April (T_{Apr}) and monthly rainfall in November (R_{Nov}) significantly induced pine growth in both measuring techniques ($p < 0.05$). For TA System, the results of the regression indicated that the model explained 26.9% of the ARS index variation, while the results of the regression from ImageJ measuring system indicated the model explained 27.6% of the ARS index variation. The final predictive models respectively derived from climate data with ARS Index of TA System (ARS_{TA}) and ImageJ (ARS_{ImageJ}) measuring ring width data were:

$$\mathrm{ARS_{TA}} = 2.600 + 0.063\mathrm{T_{Jan}} - 0.101\mathrm{T_{Apr}} + 0.001\mathrm{R_{Nov}} \quad (1)$$

$$\mathrm{ARS_{ImageJ}} = 2.666 + 0.062\mathrm{T_{Jan}} - 0.102\mathrm{T_{Apr}} + 0.001\mathrm{R_{Nov}} \quad (2)$$

Based on modeling the effects of temperature and precipitation on ring width explained by Fritts (1976) and from equations (1) and (2), the temperature increased in the winter (January) followed by the decline in temperature in the summer (April). This reduced the water stress and stimulated the growth before the specified time at the beginning of the raining season similar to the continuous rain in early winter that kept the pine growth continuing. Pumijumnong and Eckstein (2011) and

Pumijumnong and Palakit (2020) also found a highly negative correlation between pre-monsoon temperature and pine growth in several sites of northern and central Thailand, respectively.

Ring width dataset comparisons

Ring width data measured from TA System and ImageJ software were compared by Verify for Windows and were found slightly different. Table 3 shows several comparative statistics derived from both measuring techniques. All paired-ring width series showed significant similarity of measurement since the average squared difference between the measurements was lower than the confidence level of 0.01 and the difference of average ring width from TA System and ImageJ was only 0.007 mm or 0.144%. The correlation between the 803 tree-ring widths measured by these two techniques showed a significant correlation (r = 0.99, p = 0.000) (Figure 4). The relationship between the measured ring width data from TA System (RW_{TA}) and ImageJ (RW_{ImageJ}) was:

$$RW_{TA} = 0.9987 RW_{ImageJ} \quad (3)$$

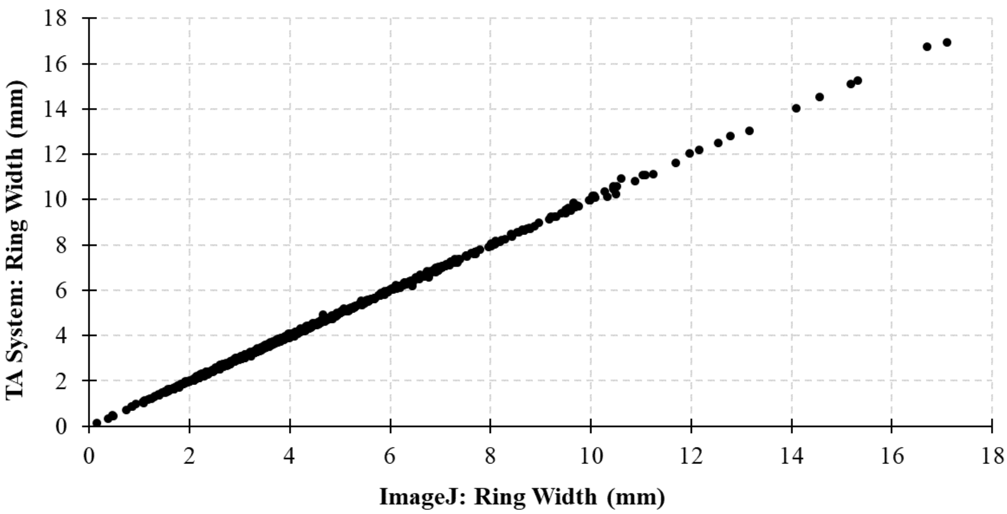


Figure 4 Correlation between ring width data measured by TA System and ImageJ.

At the confidence level of 0.01, the paired samples t-test was conducted to determine the effect of measuring systems on ring width variations. The results also showed little difference between the two systems. All paired samples of 20 sample cores and ARS indices were insignificant differences, except sample core No. 14 that showed a significant difference between the TA System (Mean = 5.137, SD = 2.516) and ImageJ (Mean = 5.152, SD = 2.524); t(36) = 2.865, p = 0.007. However, all paired samples showed significant correlations (p = 0.000) as similar as the analysis using the Verify for Windows. Maxwell *et al*. (2011) also found a significant correlation between the ring width measurements from the TA system and WinDENDRO using the Verify for Windows to confirm the accuracy of the image analysis for tree-ring studies.

Table 3 Comparative statistics of TA System and ImageJ ring width dataset.

Sample cores	Average ring width (mm)		Mean sensitivity		Autocorrelation		Average squared difference
	TA	ImageJ	TA	ImageJ	TA	ImageJ	
01	4.060±1.597	4.066±1.611	0.427	0.430	0.355	0.338	0.003
02	3.711±1.726	3.723±1.724	0.349	0.348	0.464	0.471	0.003
03	4.113±1.777	4.141±1.766	0.382	0.380	0.317	0.322	0.002
04	4.091±1.539	4.096±1.539	0.293	0.295	0.531	0.527	0.002
05	3.106±1.035	3.109±1.036	0.310	0.308	0.587	0.595	0.001
06	3.266±0.834	3.265±0.837	0.214	0.214	0.550	0.559	0.002
07	3.440±0.699	3.441±0.695	0.179	0.181	0.582	0.576	0.002
08	3.222±0.813	3.218±0.806	0.195	0.193	0.629	0.634	0.001
09	4.555±2.834	4.569±2.848	0.414	0.416	0.471	0.469	0.002
10	5.053±2.801	5.060±2.807	0.456	0.456	0.471	0.466	0.003
11	5.866±3.907	5.879±3.925	0.424	0.430	0.547	0.543	0.003
12	6.698±3.566	6.681±3.552	0.401	0.403	0.709	0.710	0.008
13	3.964±1.801	3.971±1.798	0.388	0.390	0.399	0.397	0.001
14	5.137±2.516	5.152±2.524	0.427	0.426	0.404	0.407	0.001
15	5.463±2.474	5.430±2.446	0.296	0.292	0.670	0.660	0.007
16	3.235±1.736	3.241±1.737	0.380	0.376	0.559	0.561	0.001
17	6.974±2.070	6.988±2.078	0.224	0.228	0.757	0.752	0.002
18	7.984±1.767	8.004±1.745	0.148	0.145	0.816	0.819	0.004
19	8.030±1.969	8.051±2.000	0.221	0.222	0.614	0.614	0.005
20	4.932±1.122	4.946±1.128	0.218	0.220	0.550	0.546	0.001
Mean	4.845±2.364	4.852±2.367	0.317±0.099	0.318±0.100	0.549±0.130	0.548±0.131	0.003

The results of this study suggest that the measurement of annual ring widths can be done using both techniques, depending on the availability and adequacy of instruments as well as the ease of entry into the laboratory in various situations such as the COVID-19 pandemic. Although the technique of image analysis makes it easier for researchers to consider the characteristics of annual rings through a computer screen and several people can consider them together, the sample preparation for image scanning is very important. It must be carefully prepared and polished the sample cores so that the scanned image is clearly visible enough for analysis.

CONCLUSION

Image analysis is another suitable technique for tree-ring analysis. Several years ago, specific software for image analysis such as WinDENDRO had been developed with appropriate functions to support tree-ring analysis in multi-dimension including the widths of the total ring, earlywood, and latewood and wood density using the images of wood samples directly or X-ray films. However, in the case of basic measurements such as total ring width measurement and cost constraints on purchasing many licensed software for educational purposes, the use of a free image analysis software without copyright issues such as ImageJ can be applied. From this study, it was found that the accuracy of annual ring width measurement using ImageJ software was not different from those measured by the TA system standard equipment. In addition, these annual ring width datasets used for correlation analysis with climatic data of rainfall and temperature illustrated a similarity with the analysis using the annual ring width datasets derived from the standard equipment. Although the results of these two systems were not significantly different and ImageJ software was convenient to work anywhere, the time spent for ImageJ processing is much longer than the TA system due to the additional steps for sample core scanning and ring width data formatting to the Tucson or decadal format before analysis. Therefore, it can be concluded that the ImageJ software has the potential to be used for tree-ring measurement just like other standard equipment. However, users must understand and can transform the ring width data pattern to a specific format that can be further applied to other commonly used tree-ring analysis software such as DPL, COFECHA, and ARSTAN.

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