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Solving the "Magnification Irony" in Microscope-Based Reflected Light Image Analysis of Conifer Tree Rings

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Abstract. *This paper describes a technique for allowing the magnification of a microscope to be changed and still obtain quantitative reflectance values for low-magnification reflected light image analysis of conifer tree rings. A remotely controllable digital camera is used to capture images, and a multireflectance gray standard is used to calibrate the luminance response of the camera across a range of reflectances. The imaging system was tested by measuring two conifer rings of differing widths and densities at several different levels of magnification. Upon adjusting the camera and converting gray values to true reflectances, the earlywood maximum and latewood minimum reflectances of each ring were essentially identical across all magnification settings. Total ring widths were also equal across all magnification settings. The "magnification irony" is solved for microscope-based reflected light image analysis of conifer tree rings, and reflected light image analysis of conifer tree rings should become more prominent in future dendrochronological investigations. © 2006 Society for Imaging Science and Technology.*
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INTRODUCTION

This paper describes a technique for allowing the magnification of a microscope to be changed and still obtain quantitative reflectance values from low-magnification reflected light image analysis of conifer tree rings. Quantitative reflected light image analysis of conifer tree rings, that is, the measurement and analysis of wood reflectance of individual tree rings, has been shown to have excellent potential in several applications of dendrochronology. For example, low-magnification reflected light image analysis of tree rings has been incorporated in dendroclimatology[1,2] and dendroseismology.[3]

Successful quantitative reflected light image analysis in dendrochronology is based on the idea that light reflectance of conifer rings is related to ring density.[4] Specifically, rings with high wood density (i.e., high mass per volume) appear dark to the human eye and have low reflectance (Fig. 1, left ring). Conversely, rings with low wood density appear faint to the human eye and have high reflectance (Fig. 1, right ring).

In its own right, ring density has played an important role in much dendrochronological research. For example, latewood density has been used to reconstruct past temperature[5] and precipitation.[6] However, x-ray densitometry,[7] the method for measuring ring density, is costly and difficult to do consistently well.[8] Low-magnification reflected light image analysis obviates some of the more difficult sample preparation and measurement steps of densitometry, and given that image analysis yields data that mimic density,[2] this technique of electronic imaging should be widely applicable throughout dendrochronology.

Unfortunately, reflected light image analysis, at least when using a microscope to create images, still awaits wide use in dendrochronology, in part due to a limitation that can be dubbed the "magnification irony." Namely, in spite of having zoom magnification available on microscopes, reflected light image analysis has ironically been limited to using a single magnification setting while measuring the rings of any given tree-ring sample. This limitation arose from the premise that quantitative comparison of brightness values within a tree-ring sample is legitimate only if the components of the optical configuration of the imaging system are held constant for all rings of that sample.[4] This restriction means not changing the microscope magnifica-

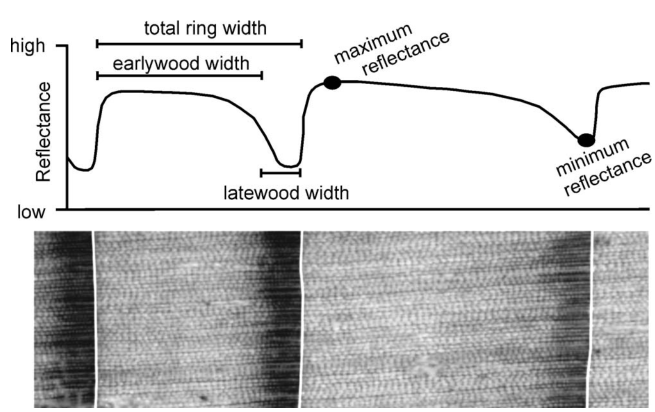

Figure 1. An image of two adjacent rings of a red spruce (Picea rubens) and their associated reflectance scans (see Ref. 4). White lines on the rings demark their boundaries, and total and partial ring widths and maximum and minimum reflectances are indicated on the scans.

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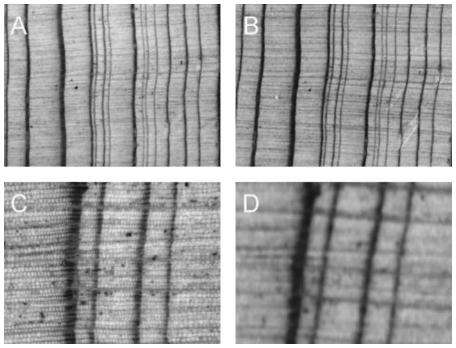

Figure 2. Comparison of images from microscope and flat-bed scanner systems. (a) Low magnification using the microscope, (b) scanned image at 1600 dpi, (c) high-magnification using the microscope, and (d) digital enlargement of the scanned image.

tion, which, when changed, dramatically affects the brightness of images.[9] This limitation is quite imposing because rings within a tree can vary by two orders of magnitude in width, for which no single magnification setting of the microscope is optimal.

Attempts to solve this magnification irony have used flat-bed scanners,[10] but it would still be useful to overcome this issue for imaging systems that use microscopes for capturing images because microscopes remain a key optical instrument in dendrochronology labs worldwide. In particular, the flexibility of zoom magnification in a microscope can be useful for samples with widely varying ring widths (Fig. 2). For example, while wide rings appear equally clear at low magnification through both a microscope [Fig. 2(a)] and a flat-bed scanner using 1600 dpi [Fig. 2(b)], narrow rings are not well resolved at low magnification. Narrow rings are clearer after optically increasing the magnification of the microscope [Fig. 2(c)], but they are pixelated after digitally magnifying the scanner image [Fig. 2(d)].

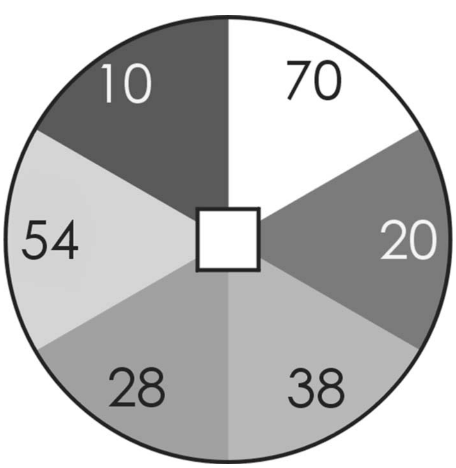

Figure 3. Multireflectance gray card standard. Each wedge is labeled here with its predetermined reflectance value (%), though those numbers are not on the actual card. The center has a white box of known dimensions, which is used to calibrate the linear distance of a pixel at any given magnification level.

A solution to the magnification irony with microscope-based imaging systems requires modifying the premise of before: Quantitative analysis of ring brightness values within a sample requires that the optical configuration be held *effectively* constant for all rings even if individual components of the system (e.g., magnification) change. This modified premise allows the components of the optical system to change as long as the ultimate effect of those changes is still a constant optical configuration. In particular, if magnification of the microscope were changed to accommodate narrow or wide rings, other components of the optical system, notably the camera itself, would have to be adjusted to compensate for the new magnification and reattain the same optical configuration of before. The primary objective of this research was to develop and automate the ability to adjust the gain and exposure settings of the video camera in response to changing the magnification of the microscope so that the optical configuration of a reflected light imaging system can be held effectively constant at different magnification settings. An additional objective was to convert measured gray value of rings to true reflectance, thereby allowing quantitative comparison of rings not only within a tree sample but also between different trees.

METHODS

Reflected Light Image Analysis System

The reflected light image analysis system used here was developed beginning with a compound microscope with a 1× common main objective lens and a zoom magnification ranging from 0.75× to 7.5× (Nikon SMZ-U). Such a microscope can be considered reasonably standard equipment for dendrochronology laboratories.

A remotely controllable digital camera (Sony X700) is used to capture images. The sensor is a 1024×768-pixel interlined charge coupled device without a Bayer mask. This device is neither a consumer-grade video camera nor a high-end scientific imaging camera, but rather it is intermediate in quality and suitable for research-vision applications. This camera has the IEEE 1394 firewire interface, which allows communication between the camera and controlling software. Gain and exposure settings determine the analog video response signal to the light input, which defines the image before being converted to digital form and displayed for quantitative analyses.

The process of computationally changing the camera's settings requires a multireflectance gray standard to calibrate the luminance response of the camera across a range of reflectances (Fig. 3). A total of six different reflectance standards was deemed sufficient for this task, with the standards ranging from 10% to 70% reflectance, approximately the same range of reflectance of typical conifer rings. All of the gray standards must be visible to the camera at the same time regardless of the magnification setting, so a pie-chart pattern was chosen with the center point positioned at the center of view of the optical system.[11] Additionally, a square of known dimensions was overlain onto the standard to calibrate the linear pixel size for any magnification setting. During each calibration step, the width of the white box is determined in pixels, and later that pixel distance is used to determine the width of the rings.

A personal computer is used as the computational platform for image acquisition and manipulation. Coriander software was selected for use within the Linux operating system. Modifications have been made to the Coriander open-source code to accommodate control of the camera settings. The software that we developed adjusts the camera by changing the exposure setting and evaluating the relationship between the measured gray values and known reflectances of the multireflectance standard. At any magnification level and for levels of illumination that are appropriate for displaying an image, this relationship can be expressed as a routine regression model:

$$\hat{y} = a_m + b_m x, \tag{1}$$

where $\hat{y}$ is the predicted gray value, a and b are the y intercept and slope for any given magnification level m, and x is the true reflectance. The camera exposure setting is changed (after converting to physical units) iteratively for each new magnification level as follows:

$$\text{exposure}_{\text{current}} = \frac{b_{\text{prior}}}{b_{\text{current}}} \times \text{exposure}_{\text{prior}}, \tag{2}$$

until a minimum difference of 0.01 is attained between b_{prior} and b_{current}.

Acquiring Images

In this application, all digital images, including the multireflectance gray standard and actual tree rings, are made and then flat-field corrected using an image of an all-gray standard.[4] The flat-field correction is done for each pixel as follows:

$$\text{corrected image}_{x,y} = \text{original image}_{x,y} \times \frac{\text{average all-gray value}}{\text{all-gray image}_{x,y}}, \tag{3}$$

where x and y refer to the horizontal and vertical location of each pixel of the images.

After calibrating the optical system using the multireflectance gray standard, the tree-ring sample is placed in position so that the rings to be measured are aligned as vertically as possible and progressing from left to right, as in Fig. 1. Ring boundaries are located using a differencing algorithm.[4] Measured gray values are converted to true reflectances by inverting Eq. (1) as follows:

$$\hat{x} = \frac{(y - a_m)}{b_m}, \tag{4}$$

where $\hat{x}$ refers to the estimated reflectance, a and b are the y intercept and slope for any given magnification level m, and y is the measured gray value. All rows of reflectance values for a ring are merged into a single reflectance scan, from which the earlywood maximum and latewood minimum values are identified (Fig. 1, reflectance scan). Ring width is obtained by multiplying the calibrated pixel length of the current magnification setting times the number of pixels of the average brightness scan. All data are written out to files in a format that is compatible with commonly used programs of dendrochronology.

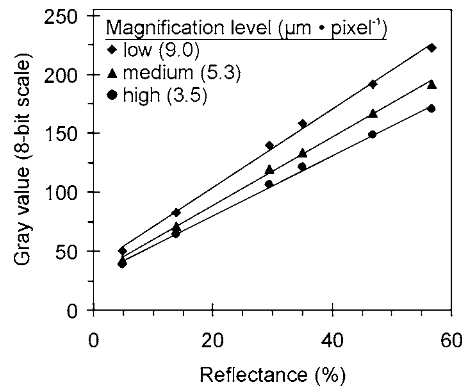

Figure 4. Scatter plots of gray values measured for each of the gray standards of Fig. 3 at three different magnification levels. The slopes and y intercepts of the regression lines differ across magnification levels because the gray value-reflectance relationship is altered by changing the magnification.

Experimental Testing

With all hardware and software components in place, the adjustable imaging system was tested by measuring two conifer rings of differing widths and densities at several different levels of magnification, with the goal of obtaining the same ring width and intraring reflectances for each ring across different magnification levels. For comparison purposes, these rings were also measured at different magnification levels but without adjusting the imaging system.

For this test, sample rings were chosen from a ponderosa pine (*Pinus ponderosa*) growing on the Catalina Mountains (2400 m elevation), just northeast of Tucson, Arizona (32° 12′ N, 110° 33′ W). Ponderosa pine is a prominent species in dendrochronology,[12] and being able to use reflected light image analysis on it would greatly enhance the use of image analysis in dendrochronology. Additionally, Tucson is within the zone of the North American monsoon, where up to 50% of annual precipitation is received during the summer.[13] Reconstructing summer rainfall in this region is vital to understanding long-term monsoonal patterns,[14] and intraring variables, including density or reflectance, have been useful for determining past rainfall patterns of the American Southwest.[1,6] Thus, successful testing of a flexible imaging system on ponderosa pine rings would pave the way for future dendrochronological research on at least the specific environmental feature of monsoonal rainfall patterns of the Southwest. In this test, the tree-ring sample was a typical increment core 5.1 mm in diameter,[15] and its transverse surface was polished with successively fining sanding papers to expose the rings clearly.[16]

RESULTS

When changing the microscope's magnification, the slope and y intercept of the relationship between known reflec-

Table I. Reflectances and widths of representative tree rings of ponderosa pine at various magnification settings.

Magnification (μm pixel⁻¹)	Camera Unadjusted Ring 1 reflectance (%)		Camera Adjusted Ring 1 Reflectance (%)			Ring 2 Reflectance (%)		
	Earlywood	Latewood	Earlywood	Latewood	Width (mm)[a]	Earlywood	Latewood	Width (mm)[a]
9.1	51	36	52	37	2.03	51	42	2.84
6.6	47	33	52	37	2.04	50	42	2.82
5.2	43	30	52	37	2.05	51	42	2.86
4.3	41	28	51	38	2.06	51	43	2.84
3.8	38	26	52	38	2.04	52	43	2.84

[a]As measured using the Bannister Incremental Measuring Machine incremental measuring machine and software (see Ref. 17), ring 1 is 2.04 mm wide and ring 2 is 2.84 mm wide. These width values are averages of five repetitive measurements of each ring. For each ring, *t* tests of ring widths show no significant difference between average values measured the traditional way versus measured by image analysis.

tance and observed gray value changed substantially (Fig. 4). Without adjusting the camera to compensate for these changes in the microscope, the measured reflectance values for both early and latewood of a test ring decreased with increasing magnification (Table I). These changes illustrate that the optical configuration does indeed vary when the microscope's magnification is zoomed in or out. Without compensating for this effect, gray values at these different configurations would differ merely because of the magnification setting, not because of any true environmental change. This would invalidate subsequent quantitative analysis of different rings measured at different magnification levels, confirming the need to adjust some other component of the imaging system after changing magnification in order to reattain the effective equivalent of the optical configuration of before.

Upon adjusting the camera, primarily its exposure setting, nearly identical gray values were obtained for the known reflectances of the multireflectance gray standard across different magnification levels. After converting gray value to true reflectance, the earlywood maximum and latewood minimum reflectances of each test ring were essentially identical across all magnification settings (Table I). Total ring widths were also equal across all magnification settings, and as verification that the widths are correct, they are the same as those obtained using a traditional incremental measuring system (Table I, footnote).

DISCUSSION

With the ability to take advantage of the magnification feature of the microscope to zoom in for narrow rings or zoom out for wide rings and still maintain a constant optical configuration, it is now easy to use a microscope-based reflected light imaging system on tree-ring samples with high interannual variability in width. The process of adjusting the camera after changing the magnification setting is automated, which makes it highly repeatable for different technicians using the system. With converting the measured brightness values to true reflectances, data from different rings can legitimately be compared across rings within a tree as well as between trees.

Furthermore, whereas in the past it had been necessary to maintain strict control over the intensity of the light source of reflected light imaging systems,[4] varying light input is no longer an issue because of this ability to adjust the camera to compensate for changes in other parts of the optical system, including the light source. Because the computerized adjustment of the camera typically takes just a couple seconds to execute, there is no reason not to recalibrate the imaging system for each new image of rings, even if the magnification setting is not changed between images. In general, a typical tree-ring series of 100 rings takes between 1 and 2 h to measure using this image analysis system. This is longer than it takes to measure just ring widths using a standard incremental measuring machine, but image analysis provides much more data that can enhance the climate modeling potential of a collection of dendrochronological samples.[1,2] As an added plus, the use of freely available software to capture and manipulate images means that no single component of the imaging system used in this research is inordinately expensive. Cost should not be a limiting factor for most dendrochronology labs to develop a microscope-based reflected light image analysis system.

In addition to the magnification irony, other issues have limited the wide use of reflected light imaging systems in dendrochronology, including the problem of wood discoloration that occurs long after tree rings are formed and therefore does not represent environmental conditions at the time of ring formation.[18] Recent advances in the use of nonwhite light[10] and in chemical pretreatments of the wood itself[19] appear to be solving this issue. As these issues are solved, further testing should proceed on a full collection of tree-ring samples using microscope-based reflected light image analysis.

CONCLUSIONS

The magnification irony is solved for microscope-based reflected light image analysis of conifer tree rings. With the freedom to change magnification between rings, image analysis is now suitable for highly variable ring widths, which is common in tree-ring samples for literally hundreds of investigations ever since dendrochronology was established as a scientific discipline. With overcoming this microscope issue, reflected light image analysis of conifer tree rings should become more prominent in future dendrochronological investigations.

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