Measurement of Three-Dimensional Morphology and Surface Area of Conifer Shoots and Roots using the Desktop Scanner and Silhouette Image Analysis

ISHII Hiroaki T.* and DANNOURA Masako

Laboratory of Forest Resources, Graduate School of Science and Technology, Kobe University, Kobe 657-8501, JAPAN.

Abstract

Accuracy and relative differences between optical and digital methods of surface area measurement were assessed by comparing measurements made using an area meter and a flatbed scanner system. A conveyer-belt-type area meter (LI-3100, LICOR Inc.) was used as the optical method of measurement. The digital method combined a desktop scanner with a light-box to illuminate the sample material from above and eliminate shadows to obtain projected area of three-dimensional objects. Object shape affected accuracy of both methods. The LI-3100 under-estimated area of deeply serrated objects, whereas the scanner over-estimated area. For comparison using plant material, conifer needles and roots were measured using both methods. There was less variability in the relative difference between the two methods for *Abies sachalinesis* needles which were larger and flatter than for needles of *Picea jezoensis*. No difference between methods was observed in the surface area measurement of roots of *Cryptomeria japonica* seedlings. Both methods yielded root surface area measurements greater than the conventional method that uses manual measurement of diameter and length of root segments to calculate surface area. As an application of the light-box scanner system, we present a detailed analysis of the three-dimensional morphology of shoots and needles of *Picea jezoensis* and *Picea glehnii.*

Key Words: Desktop scanner, LI-3100, Projected area, Silhouette area, Surface area

Introduction

Measurement of plant surface area is an important and critical part of physiological research. Many physiological processes such as photosynthesis, transpiration and respiration are determined by plant surface area and units for these measurements are expressed in terms of surface area. For example, photosynthetic rate and transpiration are measured as the amount of CO_2 uptake and water transpired, respectively, per leaf area. Respiration rate of plant organs shows strong positive correlation with surface area. Nutrient uptake from the roots is also a function of root surface area. As such, it is necessary to establish efficient and reliable methods for estimating plant surface area. The surface area of flat organs, such as leaves, can be measured using belt-conveyer-type area meters (e.g., LI-3100, LI-COR, Lincoln NE). However, it is difficult to accurately measure surface area of plant organs having complex three-dimensional shape such as conifer needles, fruits, branches and roots.

Digital technology and image analysis are being applied more and more to forest research. Many researchers now use digital measurement methods for the convenience of being able to capture data in digital format and the ease of analysis on the computer. Image analysis using digital technology enables analysis of complex, irregular structures, such as conifer shoots and root systems. However, the precision and accuracy of many newly developed digital methods have not been fully assessed. In addition, comparisons with conventional techniques have not been thoroughly made.

In a recent study, Frazer *et al.* (2001) compared digital versus film fisheye photography methods for estimating canopy openness. They found that the digital camera produced blurred edges, and in many cases estimates of canopy openness obtained using the digital camera were on average 1.4 times greater than the film estimates. Frazer *et al.* (2001) advise to use caution when estimating canopy openness with the digital camera, especially under dense canopies. Another popular digital measurement method is the use of flatbed scanners to capture images for analyses. The flatbed scanner has been used for measurement of foliage area (e.g., Thomas and Winner 2000, Ishii and Ford 2001), tree-ring analysis (e.g., Abrams *et al.* 1998, Soille and Misson 2001), assessment of diseased surfaces on leaves (e.g., Olmstead *et al.* 2001) and others (Soukupóva and Albrechtová 2003). For example, Regent Instruments (Quebec, Canada) developed measurement systems and analysis software that use high-resolution desktop scanners for image acquisition (e.g., WinFolia, WinDendro, WinRhizo). Compared with conventional optical methods, such as the belt-conveyer-type area meter for area measurement or the microscope mounted on a stage micrometer for

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* Corresponding author: hishii@alumni.washington.edu

Fig. 1. The light-box-scanner system for measurement of projected (silhouette) area of three-dimensional material. A light box containing two 23-watt fluorescent bulbs (each equivalent in brightness to 100-watt filament bulbs) is placed atop the scanner bed to uniformly illuminate the background and eliminate shadows. Images were scanned at maximum contrast to obtain the projected (silhouette) area measurement.

tree-ring analysis, flatbed scanners allow the observer to easily control the measurement resolution. In addition, images can be stored in digital format, facilitating repeated analyses and sharing of data.

In this study, we present a method of surface area measurement using the flatbed scanner. In this method, silhouette images are captured using a light box mounted atop the scanner bed (Fig. 1). This method enables measurement of three-dimensional material whose surface area cannot be measured using belt-conveyer-type area meters. We applied this method to measure three-dimensional surface area of conifer shoots and roots and compared the results to conventional methods. For further application of the method, we present a detailed analysis of the three-dimensional structure of conifer shoots and needles.

Materials and methods

A light box made of white plexiglass and containing two 23-Watt white fluorescent bulbs (Hitachi EFD23EN) was placed over the flatbed scanner (ScanJet 4c, Hewlett Packard, Palo Alto, CA) in order to obtain the silhouette image while avoiding creation of shadows. The scanner resolution was set to 300 dots per inch (dpi) and the contrast setting was adjusted to the maximum value to obtain the silhouette image. The scanned images were analyzed using Scion Image image analysis software (Scion Corp., Frederick, MD). The software is Windows based and can be obtained free of charge on the internet (http://www.scioncorp.com). The same software is also available for the Macintosh operating system (NIH Image, National Institutes of Health, http://rsb.info.nih.gov/nih-image/). Scion Image automatically determines the threshold for converting captured grayscale images to a binary black-and-white image, then counts the total number of black pixels to determine area.

To test the accuracy of this method and to compare it to a conventional method of area measurement, metal calibration disks of known area (10 and 50 cm^2) were measured using both the light-box-scanner system and the LI-3100 (LI-COR Inc., Lincoln, NE). The resolution of the LI-3100 was set at 0.01 cm^2 with a scanning width of 7.5 cm. To test for the effects of irregular shape and the amount of edge on measurement accuracy, two pieces of black acetate were cut into six- and 12-point circular stars (20.7 and 20 cm^2, respectively) and measured using both methods.

Following assessment of accuracy, we applied the light-box-scanner system for silhouette area measurement to actual plant material. Fifty sets of ten needles of *Abies sachalinensis* F. Schmidt and *Picea jezoensis* Carr. were collected from the grounds of Uryu Experimental Forest, Hokkaido University (Hokkaido, Japan). Each set of ten needles was mounted on clear acetate with clear adhesive tape and measured using both the light-box-scanner system and the LI-3100 to obtain projected needle area. The mean of three consecutive measurements was used as the final value for the LI-3100. We also measured roots of *Cryptomeria japonica* D. Don seedlings grown in pots at the Kansai Branch of the Forestry and Forest Products Research Institute (FFPRI, Kyoto, Japan). Roots were dissected into small pieces, placed atop the scanner bed, flattened with clear plexiglas and measured using the light-box-scanner system. Because the root pieces were flattened and their original shape could not be maintained following measurement, a different set of samples was measured using the LI-3100. The projected area measurements obtained using both methods were multiplied by π to obtain root surface area. In addition, root surface area of a separate set of samples was also measured using the conventional technique proposed by Karizumi (1974). Diameter and length of hand-selected root samples were measured to the nearest 0.01 mm using digital calipers (CD-20C, Mitsutoyo Corp., Japan) and root surface area was calculated as the surface area of a cylinder. Following all three types of surface area measurement, each set of root samples was oven-dried until constant weight was reached and weighed to obtain dry mass. Surface area measurements using the three different methods were compared in relation to root dry mass.

Finally, we used the light-box scanner system for three-dimensional analysis of shoot and needle surface area of one-year-old shoots of *Picea jezoensis* and *Picea glehnii* collected from a 27-year-old plantation at the Hokkaido Branch of FFPRI (Sapporo, Japan). Shade shoots that developed under similar light conditions were sampled from the lower crown of trees growing on brown forest soil (see Ishii *et al.* 2003 for a detailed description). Intact shoots were laid with the upper side facing down on the scanner bed and shoot silhouette area (SSA) was measured at 300 dpi. Then, needles were removed from the shoot twig and laid out without overlap on the scanner bed, flattened with clear plexiglass and scanned at 300 dpi to obtain projected needle area (PNA). Five representative needles were then selected from each shoot for measurement of needle cross sections. Needle cross sections were laid on the scanner bed and scanned at 1200 dpi. The cross-sectional image was analyzed using Scion Image to obtain the width and perimeter of needle cross sections. This relationship was then used to convert projected needle area to total needle area (TNA) using the following regression equation:

$$TNA = a\,PNA + b. \quad [1]$$

Where, a and b are parameter estimates obtained from the relationship between width and perimeter of needle cross sections.

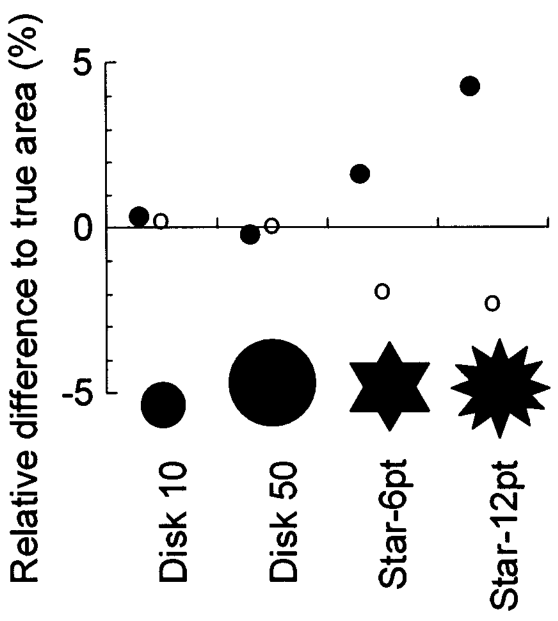

Fig. 2. Comparison of projected area measurements of material of known area using the light-box-scanner system (filled circles) and LI-3100 (open circles). Calibration disks for the LI-3100 (10 and 50 cm^2) and six- and 12-point stars made of black ascetate (20.7 and 20 cm^2, respectively) were measured using both methods.

Results and discussion

Assessment of accuracy

Measurements using the light-box-scanner system over-estimated true area of the 10-cm^2 calibration disk by 0.40% and the LI-3100 over-estimated by 0.17% (Fig. 2). The scanner under-estimated true area of the 50-cm^2 calibration disk by 0.16% whereas the LI-3100 over-estimated by 0.05%. In all cases, relative error was less than 1% indicating that both methods have a similar level of accuracy. The scanner over-estimated area of the six- and 12-point stars by 1.7% and 4.3%, respectively. Whereas, the LI-3100 under-estimated area by 1.9 and 2.3%, respectively. The scanner seems to fill-in the spaces between serrations resulting in over-estimation, while the LI-3100 does not seem to pick up the narrow points resulting in under-estimation. These results indicated that object shape affects accuracy of both the scanner and the LI-3100, but in opposite directions. From this, we infer that, large discrepancies between methods may occur for objects with more complex shape, such as deeply serrated leaves and complex branches.

Application to plant material

The scanner generally yielded greater estimates of conifer needle area than the LI-3100 for both *A. sachalinensis* and *P. jezoensis* (Fig. 3, paired t-test: t = 11.64, $P < 0.001$ and t = 7.32, $P < 0.001$, respectively). The relative difference between the two methods was -1.7% to 6.9% (scanner relative to LI-3100) for *A. sachalinensis* needles and -3.5% to 10.5% for *P. jezoensis* needles. Needles of *P. jezoensis* (0.08–0.12 cm^2 per needle) were smaller than those of *A. sachalinesis* (0.26–0.39 cm^2 per needle). As indicated by the above results on serrated objects, material consisting of smaller pieces resulted in larger relative discrepancies between the two measurement methods.

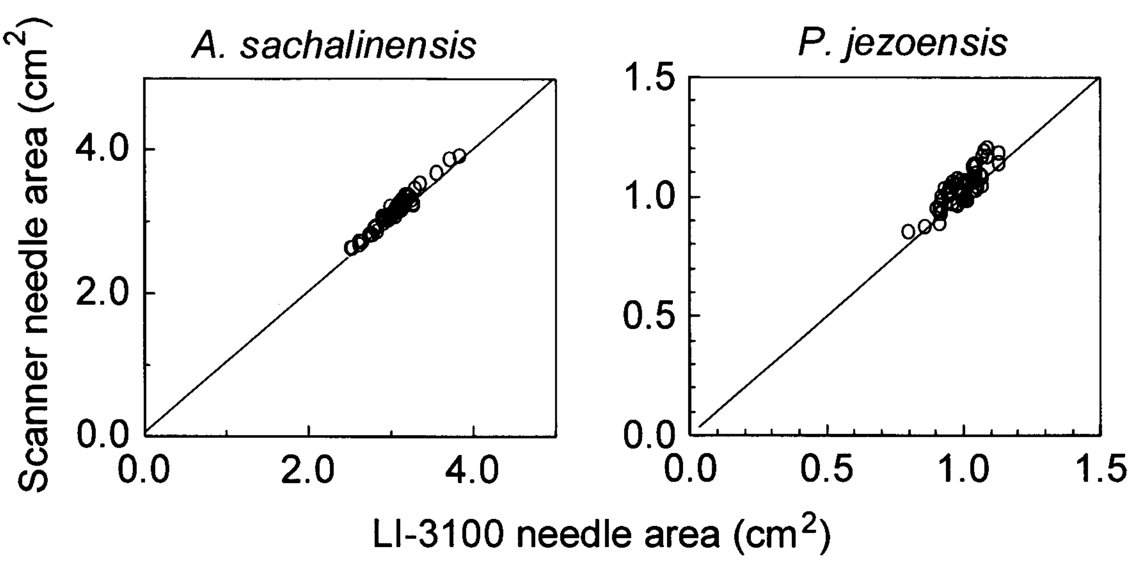

Fig. 3. Projected area measurement of ten needles of *Abies sachalinensis* and *Picea jezoensis* using the light-box-scanner system relative to the LI-3100. The line shows a one-to-one relationship.

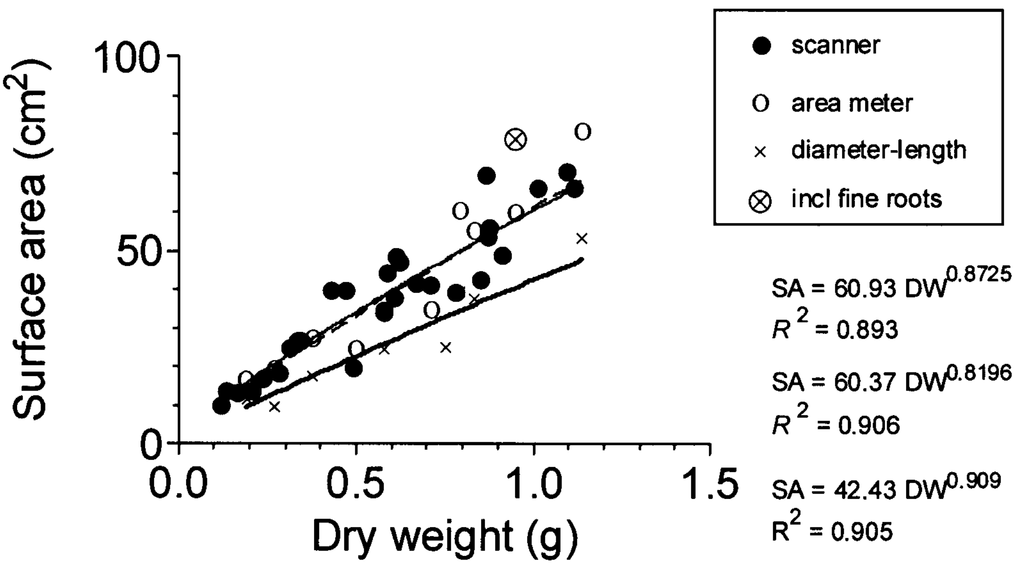

Fig. 4. Comparison of relationships between root surface area and dry mass. Root surface area was measured using the light-box-scanner system (filled circles) and LI-3100 (open circles). In addition, manual measurements of diameter and length were made on hand-selected roots and surface area was estimated by approximating roots as cylinders (x). Roots with diameter < 0.5 mm were purposively included for one measurement (⊗).

The scanner and LI-3100 yielded similar relationships between surface area of roots relative to dry weight (Fig. 4, ANCOVA, $F = 0.008$, $P = 0.930$). Surface area obtained by manual measurement of diameter and length were smaller than for the above two methods ($F = 14.88$, $P < 0.001$). This may have been because manual measurement of fine roots is difficult and sampling tends to be biased toward large-diameter sections. When roots with diameter less than 0.5 mm were purposively included, the surface area estimate increased to near scanner and LI-3100 values.

Three-dimensional analysis of shoot and needle morphology showed that shoot silhouette area increased linearly with increasing projected needle area in both *P. jezoensis* and *P. glehnii* (Fig. 5A). The slope of the relationship was similar between the two species. Similarly, the slope of the relationship between width and perimeter of needle cross sections was similar between species (Fig. 5B). As a result, the slope of the relationship between total needle area and shoot

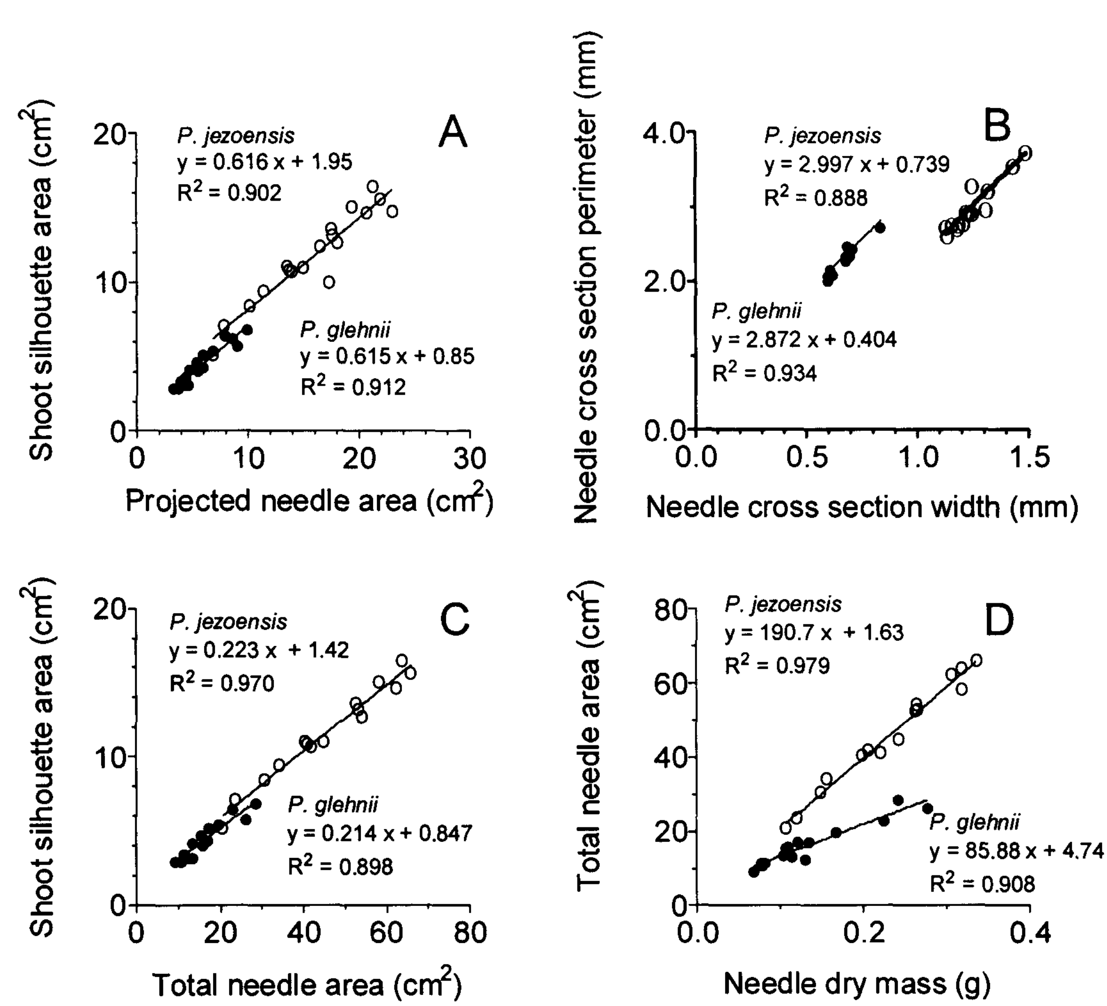

Fig. 5. Three-dimensional analysis of shoot and needle morphology. Shoot silhouette area and projected needle area of *Picea glehnii* (filled circles) and *Picea jezoensis* (open circles) shoots were measured using the light-box-scanner system (A). Needle cross sections were then taken and needle width and cross-section perimeter were measured (B). This relationship was used to convert projected needle area to total needle surface area. The relationship between total needle area and shoot silhouette area (C) is an indicator of the efficiency of display of needle surface area within shoots. The relationship between total needle area and needle dry mass (D) reflects the allocation pattern of needle mass to photosynthetic surface area.

silhouette area was similar (Fig. 5C). The relationship between total needle area and shoot silhouette area measured here corresponds to STARmax as defined by Stenberg *et al.* (1995) and is an indicator of the efficiency of needle area display within shoots. Our results indicated that the efficiency of display of needle area within shoots was similar between the two *Picea species*. However, total needle area in relation to needle dry mass was much greater for *P. jezoensis* than for *P. glehnii* (Fig. 5D). This was largely because *P. jezoensis* had longer and flatter needles than *P. glehnii*. This indicated that, in terms of needle morphology, *P. jezoensis* has more efficient allocation of needle mass to realize a given needle surface area than *P. glehnii*. Ishii *et al.* (2003) compared shoot and needle morphology between *P. jezoensis* and *P. glehnii* and found that *P. jezoensis* showed greater plasticity in needle morphology in response to changes in soil nutrient condition, especially in terms of needle length which increased significantly under nutrient-rich conditions.

Conclusions

The light-box-scanner method is useful for measuring plant material that have three-dimensional structure and cannot be measured using conveyer-belt-type area meters. However, some caution is necessary as to the accuracy of the measurement, especially for plant material with complex shape, such as deeply serrated leaves, complex branches, as well as for material that consists of numerous small pieces, such as conifer needles. Morphological complexity and the size and number of the sample material affect measurements made using the scanner and the LI-3100 in opposite directions, such that the scanner tended to over-estimate area for more complex shapes compared to the LI-3100. Both the scanner and LI-3100 yielded comparable results for measurement of root surface area. Hand-selected roots tended to be biased toward large-diameter samples and yielded smaller estimates of surface area than the scanner and LI-3100. These tendencies need to be considered when comparing surface area measurements made using different methods.

The light-box scanner system of silhouette area measurement can be used for detailed analysis of the three-dimensional structure of conifer shoots. The efficiency of needle area display within shoots and allocation of needle mass to photosynthetic area can be quantitatively compared using this method. Silhouette area measurement is a useful method to quantify the three-dimensional morphological plasticity of conifer shoots in response to changes in light and nutrient conditions.

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