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Wood Anatomy Manual for Investigation of Tree Species Grown in Ethiopia

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Abstract: Quantitative wood anatomy investigates quantitatively the variability of anatomical features in the xylem of trees, shrubs and herbaceous species. This is related to plant functioning, growth and environment and often explores how these relationships change over time. Xylem performs a wide range of functions that are essential for plants to grow and survive. Fiber length, cell diameter, cell wall thickness and lumen diameter are some of the anatomical futures which are investigated in wood anatomy. Fiber length is the distance from one end to another end whereas cell diameter is the diameter of fiber measured from side to side end and it is usually measured across the fiber length. The cell lumen diameter is the diameter of the internal cavity. It is determined by averaging the radial diameter and tangential diameter. Cell wall thickness of wood fiber is defined as half values of double wall thickness including compound middle lamella. Investigation of wood anatomy is very important that can give novel and mechanistic insights into the relationships between anatomical characteristics and potential utilization of woody and non-wood lingo-cellulose species for different industrial applications.

Key words: Anatomical Features · Cell Wall Thickness · Cell and Lumen Diameter · Fiber Length · Wood Anatomy · Xylem

INTRODUCTION

Woody species generally grouped into two types namely hardwoods (angiosperm) and softwoods (gymnosperms). Gymnosperms have a comparatively simple cell structure and are more uniform in appearance than hardwoods. Wood as a series cemented tubular fibers or cells has the main function of transporting water and nutrients, store nutrients and give support to the growing biomass of the tree [1-3].

The formation of new wood takes place in three growths related developmental stages: cell division from the vascular cambium, cell expansion (elongation and radial enlargement) and secondary cell wall deposition. The next two stages transform functional cells into dead inactive tissues and comprises of cell death and heart wood formation [3, 4].

Anatomical variations in the cell structures results in variations in wood properties within and between trees [5]. These wood properties also depend on genetic variation, growth conditions and environment [1]. Within tree differences are mostly between juvenile, heartwood and sapwood, variation within annual rings and between reaction and normal wood [5].

Anatomical characteristics have strong influences on different characteristics and appropriate utilization of each timber, bamboo and other. Wood and wood composite products such as, pulp and paper, fiber board and particle boards etc are influenced by the various anatomical characteristics such as fiber length, fiber and lumen diameter and cell wall thickness. For instance, in pulp and paper industries long fiber can have more fiber joints and therefore create a stronger network compared to a shorter fiber, this due to the longer fibers tendency to form more or bigger flocs compared to shorter fibers. The thickness of the fiber wall plays a crucial part of the paper characteristics, both in the pulping process and later on the paper process, a fiber with thinner cell wall will collapse more easily than a fiber with thicker cell wall and also these anatomical characteristics can help to predict the pattern of climate change.

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More than 70 commercial lumber tree species in Ethiopia have been investigated for their fundamental characteristics and rational utilization technologies [6]. However, the anatomical characteristics of these species mostly not investigated. This entails preparation of practical guiding manual.

Therefore, the objective of this study is to highlight all sequential steps from sample collection to anatomical sample preparation and high quality data generation. The study presents different instructions and procedures as guidance for those who are involved in wood anatomy study.

Procedure from Sample Collection to Anatomical Data Analysis and Generations

Site Selection: Site selection is crucial before collecting samples since site variations have significant effect/ influence on the anatomical characteristics of wood. Site which is representing the whole plantation area have to be assessed and identified. Aspects to be considered during site selection are: Slop of the area, Altitude, Soil type, Climatic condition of the area (Temperature, rainfall and wind direction).

Sample Collection in the Field:

- Representative sample plots from the population should be taken and all trees in the plots with good morphological quality, straight trunk and no disease or pest symptom will be selected and coded.
- The total height and diameter at breast height of all trees in the plots will be measured
- Then a minimum of six sample trees should be randomly taken from the selected plots and merchantable height and diameter at breast height (DBH) of each tree in the plot should be measured and recorded [7].
- The sample trees should be cut at stamp height of 30 cm above the ground
- Wood samples will be taken from stem discs 10-40 mm thickness obtained with a chainsaw, whereas in branches and smaller plant stems and/or root collars the entire samples should be processed.
- Sometimes Samples used for investigation of wood anatomy will be taken with an increment borer. When collecting sample by increment borer, it is even more crucial to check the sharpness of the cutting edge of the borer's tip to avoid cracks in the samples. This can be tested by punching out paper circles from a newspaper [8].
- Furthermore, it is very important to core in an exact radial direction, from the bark toward the pith, perpendicular to the axial direction of xylem cells and keeping the borer in a fixed position while drilling. The use of a pusher is recommended when collecting cores for anatomical analyses. Cores of 10-12 mm in diameter are preferable compared to the standard 5 mm or smaller, to have more material to work with and to minimize the risk of fractures and twisting [8].
- Then, coding of each sample and transporting for laboratory work [7, 9].

Preparing Micro Sections for Cell and Lumen Diameter and Cell wall thickness Determination

General Procedure*:* The following procedures will be following for the determination of, cell diameter, cell lumen diameter and cell wall thickness;

- Each sample /sample discs will be sanded by using sanding machine (Refer Figure 1a & b)
- Discs with visible reaction wood will be excluded and region close to knots will be avoided for the sampling
- If the growth ring is clearly visible we can take small blocks of wood sample from each growth ring (from each early and latewood). However, if the growth ring is not clearly visible we will take small block of wood section (20 mm * 10 mm * 10 mm) from the pith to the bark
- Clearly label, site, species name, tree number, tree position along height and also age of the sample trees.
- Boiling or just soaking the small block of wood samples in warm water (below 100°C for one hour), embedding in paraffin, or using corn starch solution often helps to avoid damage to cell structures when cutting [10, 11]. (Refer Figure 1c)
- Then 10-20 ìm wood sections should be cut cross sectionals with a microtome from each small block of wood sample [8]. (Refer Figure 1d)
- The first 3 sections will discarded to avoid cell deformation from the block cutting and then the following three sections should be taken.
- Staining of the pallid slices with an agent as safranin solution (1 g of safranin adding into 100 ml of water), Astra blue, toluidine blue, cresyl violet acetate and their combinations to increase contrast in an anatomical slide for 1-3 min [11, 12].
- Then, immersed the slice in to 30%, 50%, 70%, 90% and 100% alcohol for 3-5 min
- Leave the slice into xylen for 1 min

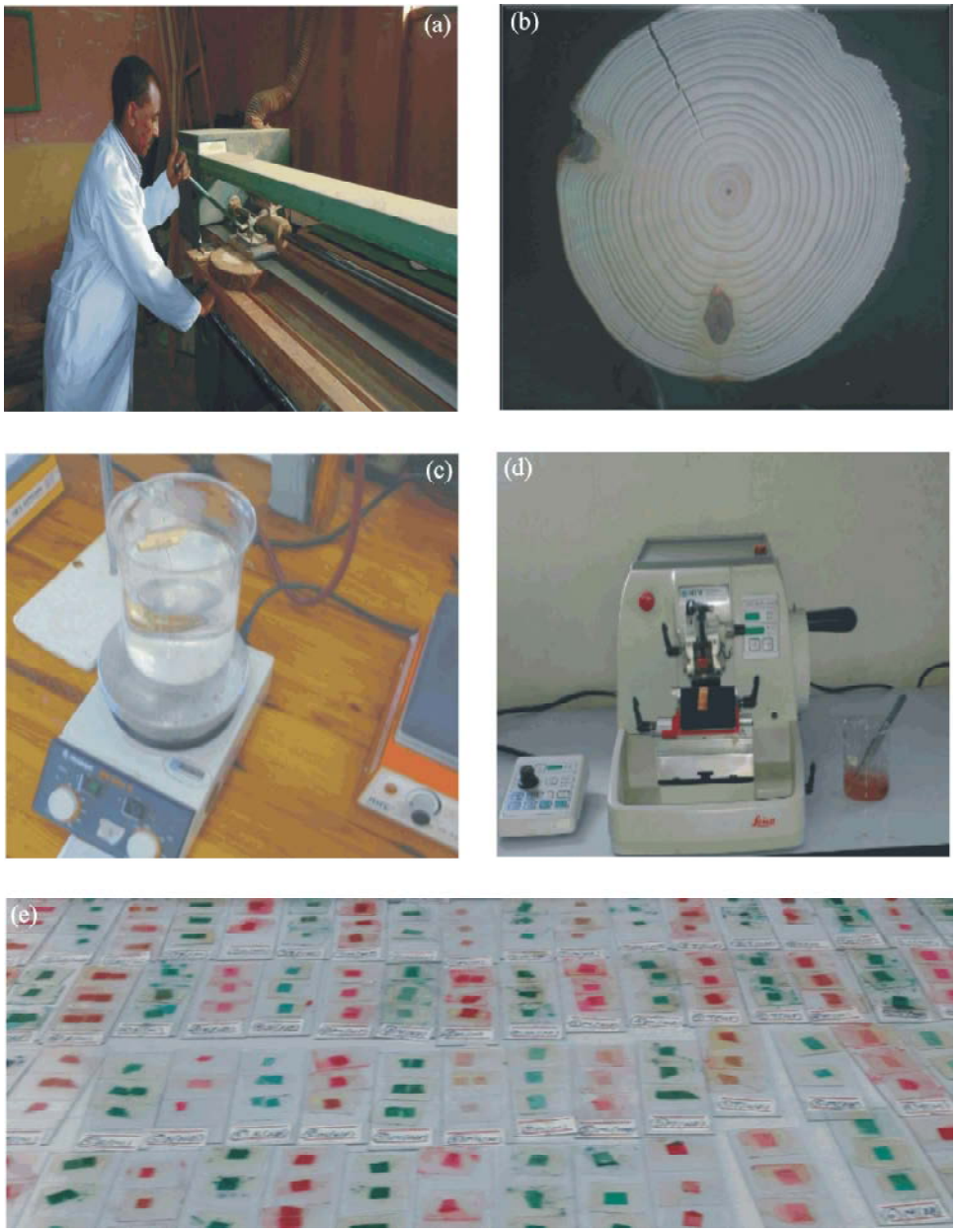

Fig. 1: a) Sanding of disc, b) sanded disc, c) softening of blocks of wood, d) cutting of micro- section, e) Prepared Permanent slide (Photo by Daniel and Lamesa, 2019).

- Next put the slice onto the microscope slide and drop small amount of Canada balsam, Euparal or Eukitt to permanently fix the sections in the glass slides [12] and cover it by using cover slip (Refer Figure 1e)
- Canada balsam and Euparal require drying in the oven at 60°C for 12h. Once permanent slides, prepared, it can be used over and over again and stored for longer period of time.

Microtome Blades: Microtome blades must be sharp to avoid disrupting the delicate anatomical structures. Frequent replacement or use of a previously unused part of the blade can avoid this problem. Furthermore, using high-quality blades can significantly reduce cutting time. For both gymnosperm (softwood) and angiosperm (hardwood) samples, good results were reported when using Leica 819 low-profile blades and Leica DB80 LX (Leica Biosystems, Wetzlar, Germany) and Feather N35HR and N35 blades [13, 14].

Fig. 2: Measuring of cell wall thickness, cell and lumen diameter (Photo by Lamesa, 2018)

Fig. 3: Cutting of discs with the band saw (Photo by Daniel, 2011)

Measuring of Cell Wall Thickness and Lumen Diameter: After making of permanent slide, one image will be acquired each of the three section with a camera attached microscope (Refer Figure 2).

- The images will be magnified 400x
- The cell wall thickness, cell and lumen diameter of 100 cells (as many as possible) per samples will measure by using different measuring software (Motic image measuring software version 3.2, image j, Win cell, Roxas, Laica etc)
- Then the average lumen diameter and cell wall thickness will be determined.

Determination of Fiber Length

Sample Preparation: The following Procedures should be followed for sample preparation for the determination of fiber length.

- Sanding and cleaning of the disc using sanding machine until growth ring is visible (Refer Figure 1a & b)
- The disc will be cut with the band saw into half (Refer Figure 3)
- From this half disc a thin stripe will be cut from the pith to bark (Refer Figure 4)
- From this stripe match stick size (1cm × 0.2cm × 0.2 cm) will be cut from pith to bark [9]
- Site, species name, tree number, position along height and age of sample tree should be clearly labelled
- If the growth ring is clearly visible, match stick size of wood sample from each growth ring (from each early and latewood part) will be taken
- Discs that contained compression wood should be cut on the tension side, because on this side the rings are wider.

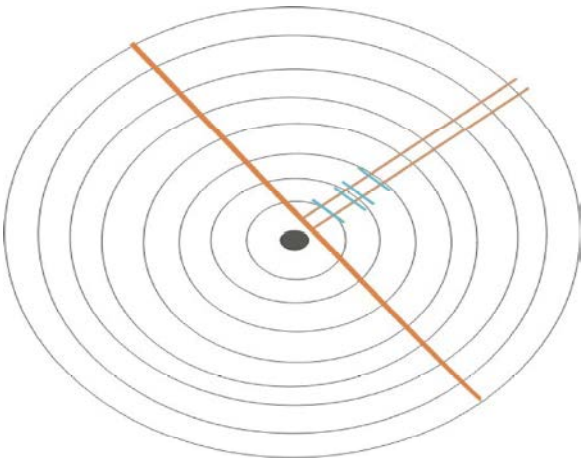

Fig. 4: Cutting of a thin strip of wood from pith to bark (Photo by Daniel, 2011)

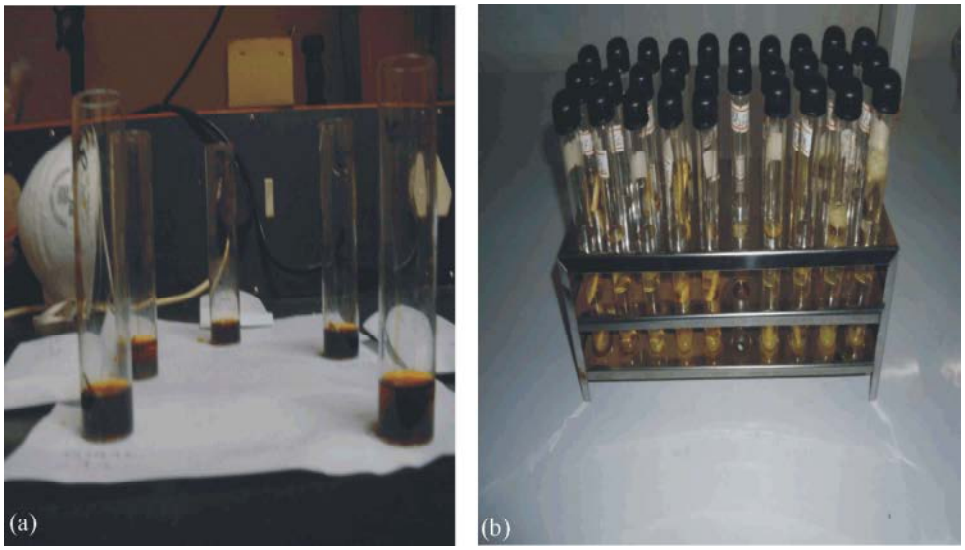

Fig. 5: a) Fiber maceration, b) Washed fibers in distilled water [Photo by Daniel and Lamesa, 2019).

- For maceration process, match stick size of wood will be macerated in Jeffrey solution (which is 10g chromic acid and dissolve it in 190 ml distil water to which 15 ml nitric acid will be added) at room temperature for 24 hours [9] or 50% nitric acid may be used since it is the most economical and less time taken method [15] (Refer Figure 5a).
- According to Mahesh, Kumar and Ansari [15] matchstick-size samples will be placed in test tubes and immersed completely in nitric acid solution and keep in a water bath at 70ºC for 5 to 6 hrs to get separated white coloured fibers.
- Then macerated fibers will be removed from water bath and allowed to cool at room temperature.
- After cooling, nitric acid will be drained and wash with distilled water and filtered using What man Grade 1 filter paper for separation of fibers [15].
- The clean fiber will be stored in fulken tube with distilled water (Refer Figure 5b).
- For slide preparation, fibers will be stained with 20% safranin solution and again washed with distilled water to reduce excess safranin.
- Finally, some amount of fiber suspension will be placed on the glass slide (standard 7.5cm×2.5cm) with the help of ink/medicine dropper and allowed for air drying [15].

Measuring of Fiber Length: The Procedures for the measurement of fiber length as follows:

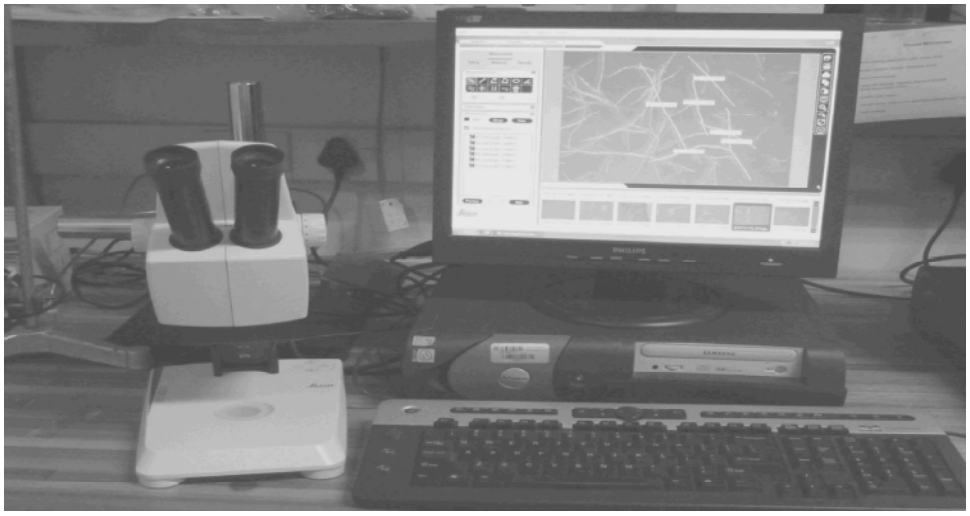

Fig. 6: Measuring of fiber length (Photo by Daniel, 2011)

- Two slides should be prepared per sample
- For microscopic analysis a few drops of the fiber suspension should be spread on a microscope slide and the water will be allowed to evaporate
- From each slide three images will be acquired with a camera attached microscopes (Refer Figure 6).
- The images will be 10x magnified [16].
- The length of 100 intact fibers (or as many as possible) will be determined with the image software [9] or 50 fibers will be measured based on [17]. (Refer Figure 6).
- Then the average fiber length per sample will be determined.

Image Analysis Tools: Once the image is produced; image-analysis tools are used to quantify the anatomical features. While target structures can be outlined and measured manually or automated image analysis allows quantifying a larger number of anatomical features in a much shorter time and in an objective and reproducible way. Several image-analysis tools are used for quantitative wood anatomy. They differ considerably in functionality, ranging from rather general image analysis software such as Image J to very specialized tools such as Motic, Las, Wincell and Roxas. The choice of the most appropriate tool depends on the specific needs. For a general characterization of xylem anatomical features in rather small samples a general tool is sufficient. However, if the sample depth in terms of number of trees, years and anatomical features measured, but also the requirements in terms of specific and comprehensive output are important for the subsequent inferences, we recommend using specialized tools. Generally, to quantify anatomical features in anatomical images, determining image resolution, image processing, image segmentation, detecting and measuring anatomical features, improving accuracy of anatomical feature detection using filter, manual editing, data storing, quality control are very important.

CONCLUSION

Wood anatomy requires high-quality, high-resolution and prepared anatomical samples. In this practical guide line, we provided good guidance to successfully investigate wood anatomy in wood science research mainly for our research center. Producing wood anatomical data is a challenging multi-step approach from sample collection to image analysis. In wood anatomy investigation, errors in one step propagate to the next step. Negligence of following standardized procedures in terms of cutting thickness, staining and illumination settings can therefore introduce considerable measurement errors and reduce the quality of the xylem anatomical data. During image analysis the encountered measurement errors can be reduced by defining specific settings for each image and manual editing, this is subjective, often very time-consuming and generally still produces less accurate data than minimizing problems beforehand. Generally, investigation of wood anatomy is very important that can give novel and mechanistic insights into the relationships between anatomical characteristics and potential utilization of woody and non-wood lingo-cellulose species for different

industrial applications. Wood anatomy research is highly recommended in Ethiopia to cover all the lumber species grown in the Country and maximize their appropriate utilization in the industry, construction and other related sectors.

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