

Wood cellular structure evaluation using image analysis methods

Jukka Antikainen

Natural Resources Institute Finland
Yliopistokatu 6, 80100, Joensuu, Finland
jukka.antikainen@luke.fi

Abstract

Light microscopy images of wood cellular structure are used to study physical and biological characteristics of the wood. Information of the microscopic structure can be used in dendrological studies, evaluating density and porosity and even determining physical changes of the modification process. Microscopic images can also be used to identify different wood species. In many cases, the microscopic images are analyzed manually which is very time consuming (10 – 30 min per image) and usually it is not possible to include all the cells from the image into the analysis. Therefore, this study shows an implementation of fast image analysis based wood cell structure analysis method which can be used for screening large sets of microscopy images automatically. Accuracy of the method was evaluated against manual measurements and the average difference varied from 1.1 % to 6.4 % for cell diameter and 5.2 % for cell wall thickness determination depending of the used parameters. Processing time for the images varied from 3 to 60 seconds depending of the image resolution and the number of analyzed cells.

1. Introduction

Microscopic images of wood anatomy are used to study growth and development process of the tree. In addition to the basic characteristics the microscopic images are used to evaluate density, appearance, water penetration resistance etc. [1] Anatomy images can be used to analyze the effect of different modification such as thermal and compression modification. [2]

Cell size and cell wall thickness information are directly proportional to the density of the wood. Large cells with thin cell walls are more porous than cells with small cells and thick cell walls. In a case of compression modification it is possible to analyze how much the actual cells have been compressed and how much the sample has been densified. [2] The analysis of the microscopic images is commonly made manually by evaluating pixel distance information of the image using Image Pro Plus [3] or other similar image analysis software. Manual inspection is very time consuming and expensive. Therefore, automatic image analysis method and implementation for large image collections is needed.

In addition to the basic manual evaluation, microscopic image analysis can be done using tools such as WinCELL [4] and ROXAS [5]. The ROXAS developed over Image Pro Plus software and it is free for scientific use. WinCELL and ROXAS are developed specially for wood cell analysis and they can be used to evaluate various parameters such as cell size, cell types etc. However, the commercial tools often require user input (region selec-

tion, pre-processing etc.) for each microscopic image separately. This process is time consuming and it can take hours for large set of images. This process should be automated and the user input can be minimized using proper selection of filtering and automatic preprocessing techniques.

Brunel et al. [6] proposed a method for automatic identification and characterization of wood cells from microscopic images. The method is also suitable for detecting different cell types and orientation.

The implementation in this study is based on the Brunel's method with some improvements and adjustments for the cell shape analysis. The main focus of this study is on fast automatic cell diameter and cellular wall thickness determination. One important point of the proposed implementation is that it does not require any user input to function properly and it can be used for multiple images and datasets at once.

2. Materials and methods

2.1 Light microscope images

A set ($n = 94$) of Scots pine (*Pinus sylvestris*) light microscope images were used. Images are used in a study where physical characteristics of same wood specie between different geographical and climate regions are evaluated. However, this paper focuses only to the image processing part and evaluates the usability and accuracy of the proposed method.

2.2. Manual image evaluation

Five images (5.4 % of 94) from two different datasets were selected for the manual evaluation. Each image was evaluated using external image processing tool by measuring pixel distances from selected two points. Diameter of the lumen (inside part of the cell) was cross measured from two orthogonal directions. Cell wall thickness was measured between two cells and the result was divided by two to obtain single cell wall. In the evaluation, almost every cell from the each image has been included into the evaluation and to the final result.

2.3 Automated image processing

The images are collected with different microscopy imaging setups. Different illumination, cameras and the imaging parameters have affected to the contrast and brightness levels and the colors were totally different between separate microscopies. Therefore, the images are preprocessed before the analysis part.

Preprocessing

At first the original image (Fig. 1) contrast is enhanced by contrast stretching (normalization). The noise is filtered using median filtering because it reserves the edges but removes the noise. The grayscale image is generated from the average of the RGB color channels.

Thresholding

The grayscale image is transformed to the black and white image (Fig. 2) using Otsu's thresholding method [8] which maximizes the between-class variance.

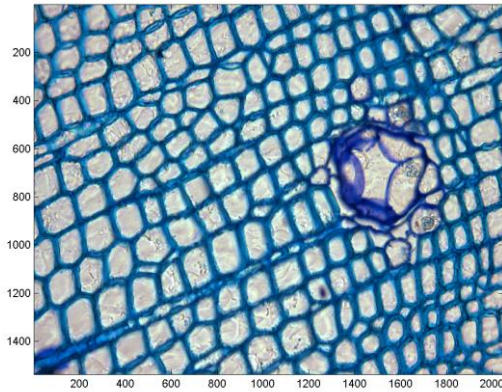


Figure 1. A light microscopic image from a Scots pine cellular structure

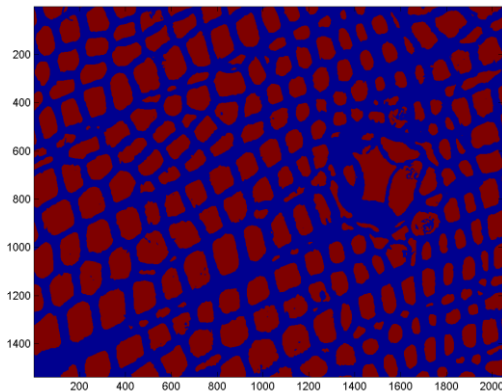


Figure 2. Binarized image from Otsu's thresholding method

Distance transform

Distance transform for the B&W image is calculated using the Euclidian distance where each pixel distance to closest nonzero variable is determined. [9] The distance transformed image (Fig. 3) is used for as an input to the watershed segmentation algorithm.

Watershed transformation

The watershed transform [7, 11] is used for segmentation of the different cells on the image. Before the watershed transform the distance image is weighted using H-minima transform [10] which reduce the count of misclassified cells. As a result of the watershed transform an image with labeled cells is generated (Fig. 4).

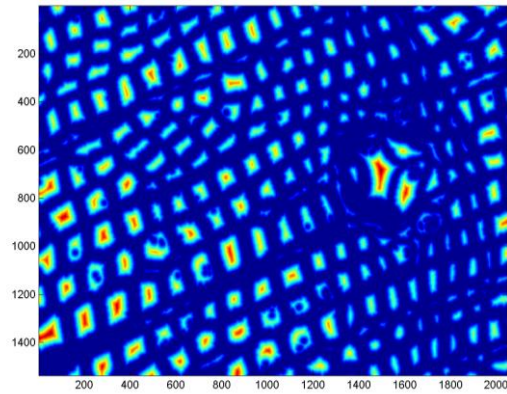


Figure 3. Distance transform image calculated from the binarized image

Region adjacency graph

Region adjacency graph is composed of nodes and edges. Nodes are representing the regions (segments) and edges are representing the connection between adjacent segments (neighbors). The labeled image from the watershed transform is used for generating the regional adjacency graph (Fig. 5) which is used for the cell diameter and cell wall thickness determination in the line analysis approach. [12, 13]

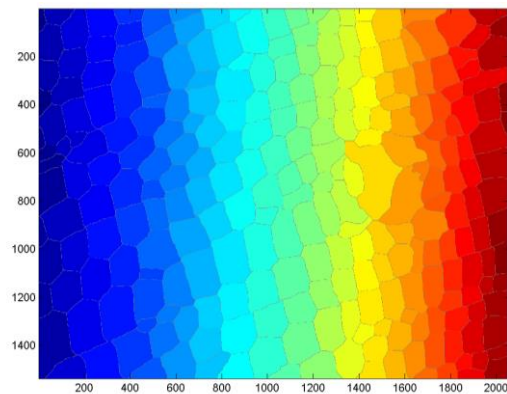


Figure 4. Labeled image generated by the watershed transform

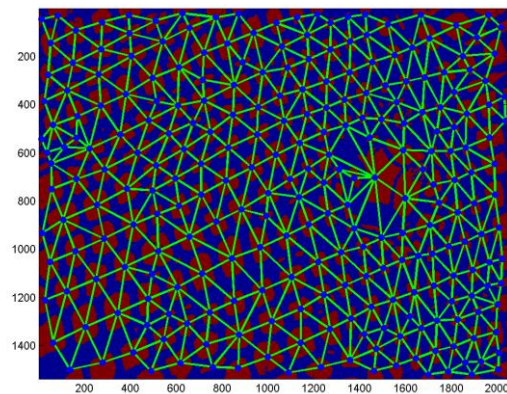


Figure 5. Region adjacency graph calculated from the labeled image

Analysis of cell characteristics

Cell characteristics are determined using line profile analysis. Line analysis is done from each edge of the adjacency graph. The starting point is from the center of a cell (lumen) and the ending point is the center of neighbor cell. Cell walls are located between the cells and they are recognized from nonzero values. Now it is possible to evaluate the amount of pixels from the center to the cell wall. Cell diameters are obtained in several directions (count of edges) which can be used to evaluate the shape of the cell. Also the cell wall thickness can be evaluated in a similar way. Because the cellular wall thickness determination includes both cell walls (own and neighbor) the calculated thickness must be divided by two. Each edge between cells is analyzed only once. Cells located at the borders of the images are removed from the analysis.

3. Results and discussion

The image processing and analysis implementation was done with Matlab and Image Processing Toolbox. Implementation was tested on HP EliteBook 8570w laptop.

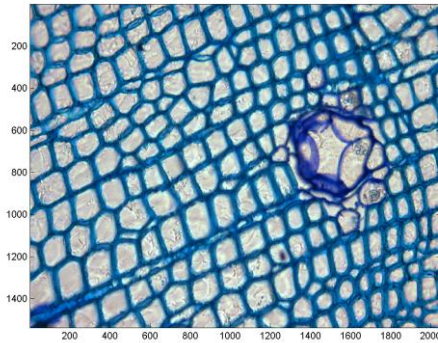


Figure 6. Example #1 microscopic image

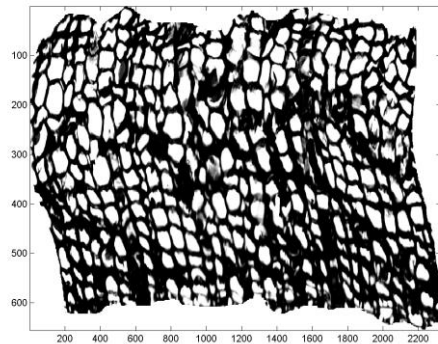


Figure 7. Example #2 microscopic image

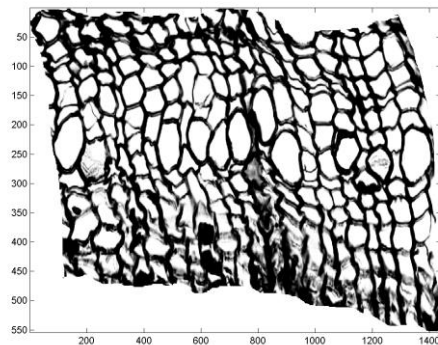


Figure 8. Example #3 microscopic image

The image analysis was tested for a several different image datasets. Some images were full and some were cropped to emphasize the region of interest. The processing time varied from 3 seconds up to 60 seconds depending of the image resolution and the number of analyzed cells. The line analysis was the most time consuming part around 60 % of total computation time.

Figures 6 to 11 shows examples of analyzed images and determined cellular characteristics. Cell number on x-axis describes the count of the edges on the adjacency graph. The result data includes some outliers (peaks and near zero values) which can be easily identified and removed from the final result. Some of the samples (e.g. Fig 8) were distorted and blurred which can be recognized from the result graph (Fig 11). In blurred parts the cellular wall thicknesses were overestimated and cell diameters were underestimated. Removing outliers using different filtering levels will clear out most of the problematic parts of the data and the average characteristic can be determined accurately enough.

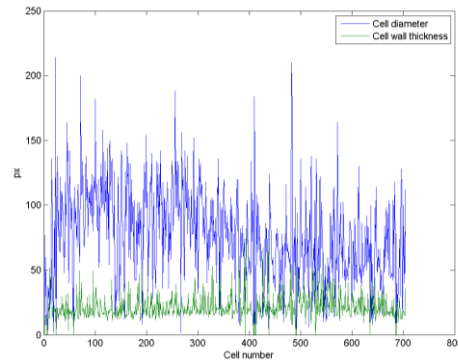


Figure 9. Characteristics for example #1

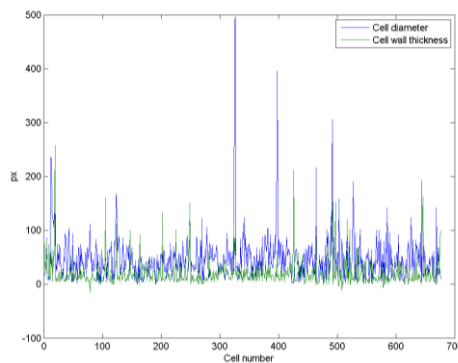


Figure 10. Characteristics for example #2

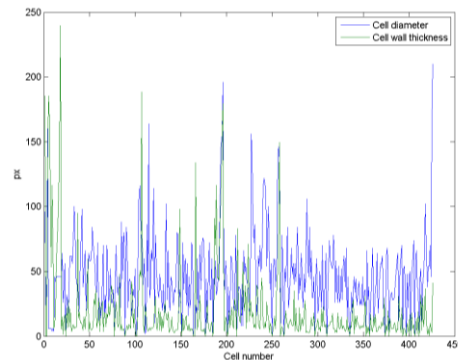


Figure 11. Characteristics for example #3

Automatic evaluation was compared to manual reference method (Table 1). The experiments were done with two different sets of filtering parameters (f_{over} & f_{under}) which are used to select proper filtering levels to remove over and underestimated cells from the final result. First parameter set (Table 1 (a)) was selected individually for each dataset and the average evaluation results were very close (**1.1 %**) to the manual evaluation. Second parameters (Table 1 (b)) were dynamically generated for each image. Filtering levels were dynamically generated using $f_{over} = average(diameter) * 1.9$ and $f_{under} = average(diameter) * 0.1$ and levels worked well for most of the images but not for all and therefore the average difference (6.4 %) was greater than with the first case.

Table 1. Cell diameter estimation using manual and automatic methods for randomly selected images. (a/b)* two different filtering parameters

Sample (dataset)	Cell count	Manual [px]	Automatic [px] (a/b)*	Difference [%] (a/b)*
1 (1)	268	53.4	54.2 / 53.7	1.5 / 0.5
2 (1)	94	54.0	53.1 / 48.2	-1.7 / -10.2
3 (2)	293	37.5	37.2 / 38.3	-0.7 / 2.2
4 (2)	262	39.2	39.4 / 42.0	0.5 / 7.0
5 (2)	255	45.8	46.2 / 51.4	0.9 / 12.2

Table 2. Cell wall thickness estimation using manual and automatic methods for randomly selected images.

Sample (dataset)	Cell count	Manual [px]	Automatic [px]	Difference [%]
1 (1)	268	7.0	7.3	4.3
2 (1)	94	10.1	9.8	-3.0
3 (2)	293	8.6	8.2	-4.6
4 (2)	262	6.7	6.2	-7.5
5 (2)	255	8.8	8.2	-6.8

Differences between cell wall thickness evaluations were relative large (Table 2). The average difference (**5.2 %**) might be result of the manual selection of the cell walls. In some cases the cell walls were very thin which were hard to measure manually from the screen. It should be noticed, that manually determined cell characteristics will contain some human errors even the selection and evaluation have been done as accurate as possible.

4. Conclusions

This paper described preliminary results of the ongoing study and development process. The proposed implementation works well for different data sets with different setups and resolutions. However, there is still some experimental work to be done with the dynamic selection of the outlier filtering parameters and this issue will be studied and improved in the future research. Also some intensive comparison between commercial analysis software will be done.

Nevertheless, the automated analysis method has already saved hundreds of hours of manual work. The analysis speed is fast enough but a parallel implementation should be considered at least for the most time consuming part (line analysis). Parallel implementation will definitely increase the performance of the whole analysis. It is also possible to add cell alignment determination and cellular type detection like described in Brunel's study [6].

In the future, the analysis method will also be imported into the microscopy with computer controlled xy-sample table. Analysis will be included as a part of the specimen imaging process which decreases the screening times even more.

References

- [1] J. M. Dinwoodie, "Timber: Its Nature and Behaviour", Taylor & Francis Ltd, 2000.
- [2] D. Dogu, et al., "Anatomical investigation of thermally compressed wood panels", *BioResources*, 5(4), pp. 2640-2663, 2010.
- [3] Image Pro Plus, Media Cybernetics home page: (<http://www.mediacy.com/imageproplus>), referred 3.3.2017.
- [4] WinCELL, Regent Instruments Inc. home page: (http://regent.qc.ca/asset_s/wincell_about.html), referred 3.3.2017.
- [5] G. Arx and M. Carrer, "ROXAS - a new tool to build centuries-long tracheid-lumen chronologies in conifers", *Dendrochronologia* vol. 32 pp. 290-293, 2014.
- [6] G. Brunel, et al. "Automatic identification and characterization of radial files in light microscopy images of wood", *Annals of Botany*, vol. 114, pp. 829-840, 2014.
- [7] Jos B.T.M. Roerdink and A. Meijster. "The Watershed Transform: Definitions, Algorithms and Parallelization", *Fundamental Informaticae*, vol 41, pp. 187-228, 2001.
- [8] N. Otsu, "A threshold selection method from gray-level histograms". *IEEE Trans. Sys., Man., Cyber.* 9, pp. 62-66, 1979.
- [9] M. Calvin, et al. "A Linear Time Algorithm for Computing Exact Euclidean Distance Transforms of Binary Images in Arbitrary Dimensions," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, Vol. 25, No. 2, February 2003, pp. 265-270.
- [10] Soille, P., "Morphological Image Analysis: Principles and Applications", Springer-Verlag, pp. 170-171, 1999.
- [11] M. Fernand, "Topographic distance and watershed lines," *Signal Processing*, Vol. 38, pp. 113-125, July 1994.
- [12] David Legland, Region Adjacency Graph (RAG), <https://se.mathworks.com/matlabcentral/fileexchange/16938-region-adjacency-graph--rag>
- [13] J. Blanc-Talon, "Advanced Concepts for Intelligent Vision Systems" in Proceedings of 8th International Conference, Acivs 2006, Antwerp, Belgium, September 18-21, 2006.