# Image Analysis for wood anatomists

WinCELL™ 2020
Analyser of Wood-Cell Structure Parameters over Annual Rings

Anatomical wood cell analysis is an alternative to wood density analysis with x-rays, as done with WinDENDRO. Wood density, color, mechanical and chemical properties are related to wood structure which in turns is related to climate. By measuring the radial cells (tracheids) size, distribution and their proportion to walls, wood quality can be assessed.

Wood cells morphological data can be measured per annual ring in images that contains more than one ring or in a series of images each containing a fraction of a ring.

Image analysers not made specifically for wood cells measurement rarely produce data suited for dendrochronology studies. They lack some knowledge about annual tree rings formation and their cells structure (to compute the ring width, for example). **WinCELL** has this kind of know-how built-in. It can analyse different wood species: vessels of deciduous and radial row of conifer tracheids.
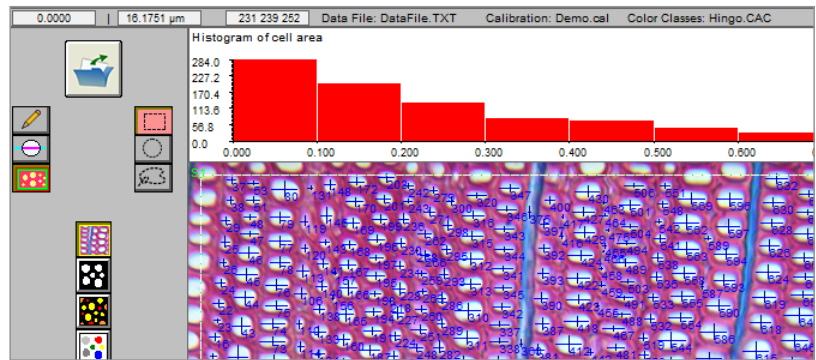
WinCELL supports different automatic and interactive analysis modes. The latter allows you to select rows of cells to analyse them in a way that mimics traditional tracheidograms (curves of radial cell size variations in function of position in an annual ring).

Digitize and Analyse Wood Cells with WinCELL™

## The First Step - Acquire images with a digital camera or a high-end scanner

Wood cell analysis is traditionally done on thin wood slices cut with a microtome. Images are acquired using a digital camera mounted on a microscope. It is interfaced to computer via a fast USB connection.

Staining is sometime used to enhance lumen-wall contrasts when acquiring images in translucent light. This is the ideal setup to acquire very high resolution and precise images but the field of view is usually limited so that it is difficult or impossible to view entire rings. Such images can be analysed and their data merged afterward in **WinCELL** to do cell analysis on a ring basis.
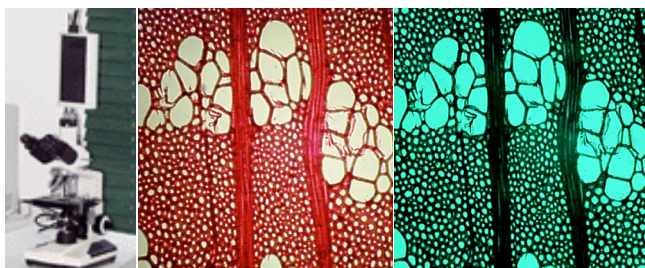
Alternative sample's preparation methods and imaging over larger areas also exist to analyse, for instance, larger cells like earlywood vessels of deciduous species. They are typically based on a scanner or a camera with macro lens and proper lighting.

Here the difficulties are to acquire images with enough contrasts between lumen and walls without damaging them and to get enough resolution to accurately identify and analyse them. These methods allow to acquire images of a few annual rings per image, making their analysis easier.
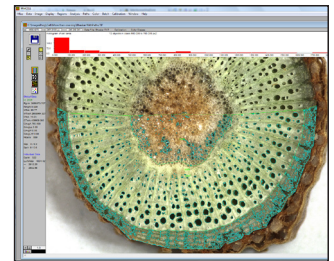
If you plan to use a scanner please inquire about the conditions before purchasing. **WinCELL** is TWAIN compatible, meaning that you can acquire images from a few camera or scanner models simply by clicking an icon in **WinCELL** main window. You can also set the image parameters (size, color, filtering) while the image is displayed.

The Second Step - Choose the region to analyse

WinCELL analyses whole images or regions of any shape, as shown on the two figures below. There can be practically an unlimited number of such regions which can overlap or not. They can include just a few cells, e.g., the vessels of a ring, a complete ring or any region you wish, such as the entire image minus some defects or bark. Their positions can be saved to be recreated on different images at a later time. Regions can be resized or moved and the analysis is updated automatically.

Ring analysis
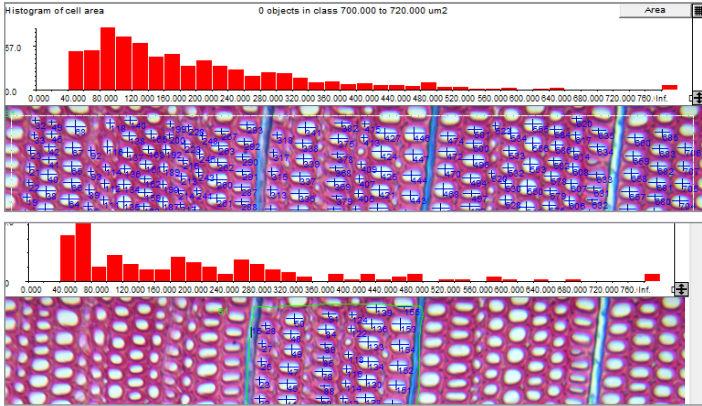
WinCELL can analyse cells per annual ring for one or more rings per image. It computes statistics on a yearly basis in its own format or in WinDENDRO's data format which is compatible with stem analysis software program XLStem.

The annual ring analysis process is semi-automatic. Ring boundaries are first identified with boundary traced and year entered, as shown below. Then **WinCELL** creates one analysed region per ring. This region has all the usual wood cell measurements, i.e., area, length, width, position, perimeter, form.... plus the ring data (area and width), and can be done interactively or in batch without operator supervision.

The ring boundaries tracing step can be done also within WinDENDRO to save time. The rings below have been detected in WinDENDRO. The analysed image was then exported to WinCELL, which created a ring analysis region.

Data available per analysis region, image or annual ring are numerous and user selectable. They include: ring width and area, number of cells, cell density, total area occupied by cells and walls, average cells area, length, width, perimeter, form coefficient and more. These measurements are explained below.

# Measurements

Lumen and Wall Area

Lumen and walls area are measured automatically and are a true measure of their area based on the number of pixels contained within, not an estimation from the cell's diameter. Lumen can be separated into cells and vessels types based on their area. A third cell type, parenchyma, is available on a manual classification basis. Cells of different types are analysed separately and you get global and individual data for them. The classification of a cell can be changed interactively.

Lumen and wall area are available as total values for the analysed region in measurement units and in percentage area. Lumen area is also available on cell basis.

In addition to cell and wall areas, the Pro version can also measure the lumen and wall area in function of their color, globally for the analysed region or individually, i.e., per cell.

Tracheid Length and Width

Tracheids length and width are available as average values for the analysed region and individually per cell. Length and width, or radial and longitudinal diameters, can be computed by the 4 different following methods:
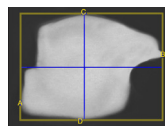
- **Maximum (Horizontal & Vertical)**

Length is measured as the horizontal distance between the two farthest cell boundary points of same vertical position. Width is the vertical distance between points on the same horizontal position.
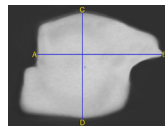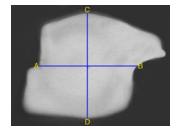

- **Bounding Box (Horizontal & Vertical)**

Length and width are measured as the horizontal and vertical size of the bounding rectangle that encompasses a cell.
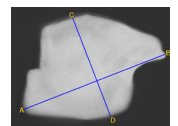

- **Center (Horizontal & Vertical)**

Length and width are measured as the horizontal and vertical size of the cell in its centre of gravity position.
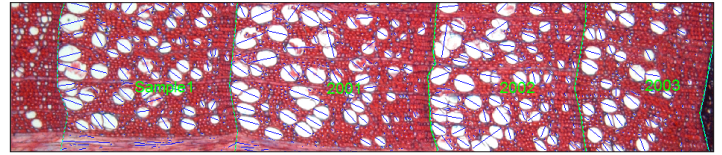

- **Any direction**

Length is the distance between the two points on the cell boundary that are the farthest apart and width is measured perpendicular to this length at the position where the width measurement is the largest. Walls are not calculated with this method.
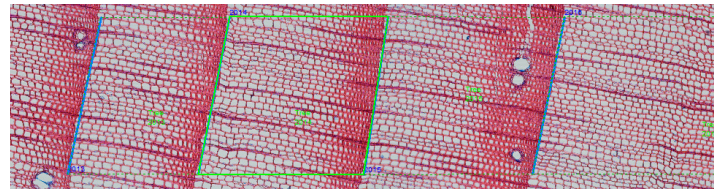

Cell Position

Cells centre position can be measured in the image and relative to the annual ring beginning. The latter allows to compute statistics about cell structural parameters distribution in function of position on annual rings.
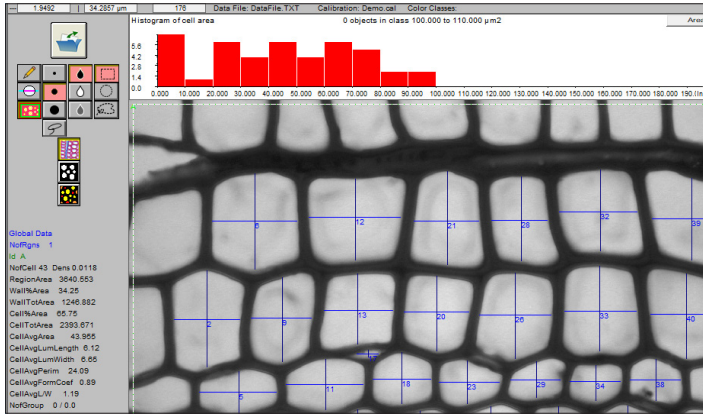
Number of Tracheids (cells, vessels and parenchyma)

Cells and vessels in an analysed region or on traced paths are automatically counted.

Interactive Measurements

The morphological measurements can be computed automatically on all cells present in an analysed region or on selected cells via interactive measurements.

In automatic mode, click the image or trace the outline of a region and cells included in that region will be analysed. In interactive mode, you must draw a line across the cells to measure. This mode is typically used to analyse radial cell rows, i.e., rows of cells which position relative to a ring boundary is known.
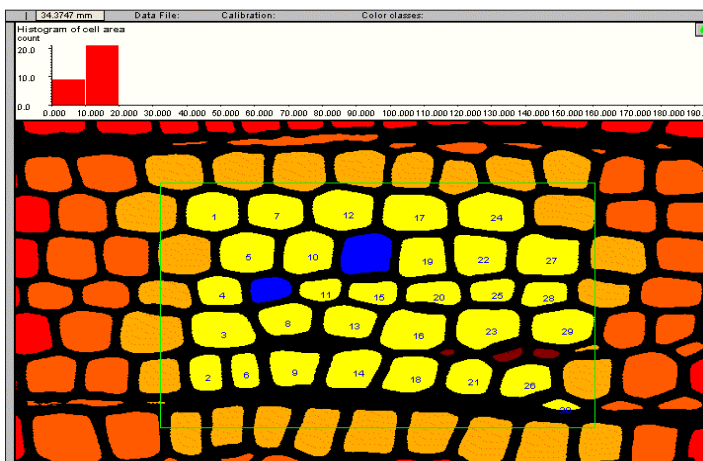

There are four types of interactive measurements:

• In manual mode, no analysis is done. It is provided to measure any object's length simply by clicking at its ends. It can be used to measure a cell length or as illustrated to the right, fibres length. Straight objects require only two mouse clicks while curved objects need additional mouse clicks at inflection points along their curvature.
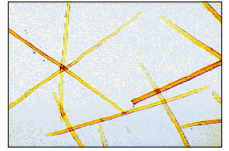
• Another manual mode is provided to measure an object length and width in pair which can later be used to estimate its area. This mode is useful to measure cells which don't have enough contrast for the automatic analysis.

• Two semi-automatic measurement modes allow to measure cells length and their two adjacent walls thickness on opposite sides of the lumen. With one method, this is done in the traced direction while with the other, this is done in the radial direction. These two semi-automatic measurement modes also produce the length of radial cell rows between annual ring boundaries to get ring width.

Features

Analyzed region

WinCELL uses the concept of analyzed region in order to reject incomplete cells. Cells touching the image boundary or located outside the analyzed region, like cells drawn in red and orange in the image below, are not considered when computing average cell measurements (area, length and width). The color used to draw a cell indicates its classification: partially or completely outside or inside the analysed region, rejected by operator, debris, cell type (cell, vessel or parenchyma).

Measurements data are available interactively during the analysis and in text files that can be read by many software programs. These files are easily opened and visualized in spreadsheet style programs like Microsoft Excel. You can also click a cell to display its morphological measurement data.

The cells distribution histogram, visible during the analysis or after in XLCell, also presents a global view of the cell structure parameters. The cells distribution histogram displays the number of cells in function of area (see image below), length or width, and the area in function of color for the Pro version (see next page).
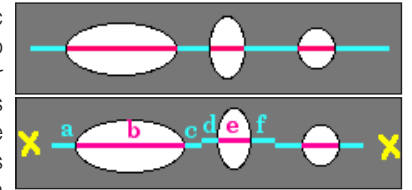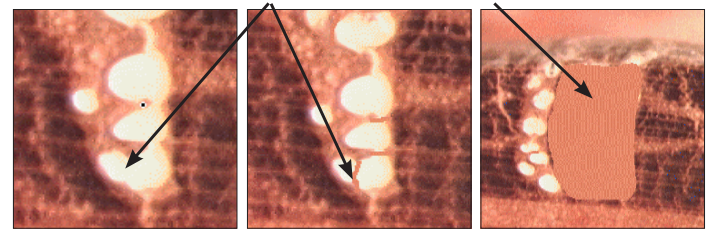

Image edition and debris filtering

Image edition allows to compensate for defects or poor contrast. Images can be edited easily with any color present in the image. A pen, to draw lines, and a lasso tool, to fill outlined regions, are provided for this purpose.

Defects or regions you do not wish to analyse can be excluded by outlining them with Exclusion Regions or by editing the image. They can be used in Ring Analysis to skip gaps or cracked areas on dried wood or broken cores.
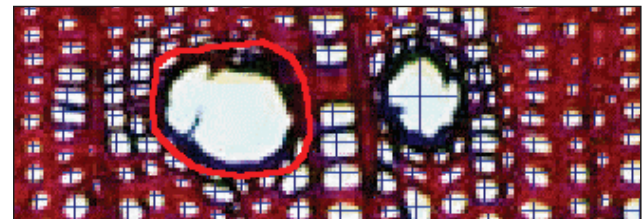
Defects or non cells objects (debris), can be automatically filtered out by morphological features (area, length, width, form, length to width ratio), color (see Pro version on next page) or by editing the images.

Continued (Features)

Cells grouping

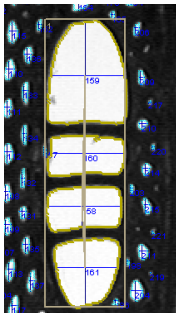Cells can be grouped, like the four parts of this vessel, to be analysed as a single one. Data are available as individual cells and as groups of cells. The number of groups and the average number of cells per group are also provided in the data files.

Batch processing

It is provided to analyse a series of images without operator supervision. This analysis mode works only for analyses that can be done automatically (non-interactively).

Analysis settings

They can be stored in configuration files for retrieving and reusing at a later time.

Image Calibration

A calibration must be done or loaded for images acquired with a camera. It is easy to perform on targets sold by microscope manufacturers. Different target models are supported by **WinCELL**.

Original images acquired from **WinCELL**

Image acquired from **WinCELL**, analysed or not, can be saved in standard *tiff* or *bmp* files for opening in other application programs. Images saved with their analysis in the same file are automatically reanalysed when reopened in **WinCELL** (useful to validate or modify a previously done analysis).

Saving options

You can choose which data are saved in **WinCELL**'s data files and their format (WinCELL or WinDENDRO).

Various object analyser

WinCELL can also be used as a general area meter to measure leaf area, for example, or a morphology analyser for other objects by modifying its default settings.

Like all Regent's software, **WinCELL** is a stand-alone program with all the built-in necessary functionality. It does not require an additional complex image analysis program or user programming skills as in some other cell analysis programs.

WinCELL™ is offered in two different versions

Regular

The Regular version performs all the above measurements on images in grey levels or in color. However, colors are processed as grey levels. Wood cells cannot be analysed on a color basis with this version.

Pro

Besides the analysis of images in grey levels, the Pro version also does analyses on color images. By using color classes and groups that you can define by clicking in the image, you can get:

1- a better classification of the pixels into lumen and wall, thus a better cell detection compares to grey levels one for some types of images

2- measurements such as area in function of color and potentially classify objects in function of it (see image on the right)

Color analysis with **WinCELL** Pro software requires images in 24 bits RGB (Red, Green, Blue) color format (8 bits per pixel per color channel) stored in *tiff* (uncompressed) or *jpeg* files.

WinCELL Features per Software Model

Features

|	WinCELL Software Model	
	Regular	Pro
Anatomical analysis and quantification of wood cells over annual rings		
Lumen and wall area	√	√
• total values for the analysed region in measurement units and percentage area, and on cell basis		
Tracheid length and width	√	√
• average values for the analysed region and individually per cell		
Form coefficient	√	√
Perimeter	√	√
Lumen and wall area in function of their color		√
• globally for the analysed region or individually per cell		
Cell analysis per annual ring for one or more rings per image	√	√
• whole image or a selected region of any shape can be analysed		
Spatial distribution to analyse variations in function of cell position on annual rings	√	√
Automatic count of tracheids in an analysed region (cells, vessels and parenchyma)	√	√
Cells touching the image boundary or outside the selected region are not analyzed	√	√
Interactive measurement modes	√	√
• manual for any object length (cells, fibres...)		
• manual for an object length and width in pair to estimate its area		
• semi-automatic for cells length and their two adjacent walls thickness on opposite sides of the lumen		
Data computed on a yearly basis in a format similar and compatible with WinDENDRO	√	√
Integrated knowledge of wood cell anatomy and annual tree-ring formation	√	√
• can analyse different wood species: vessels of deciduous trees and radial row of conifer tracheids		
Analysis of images of thin wood slices mounted on a microscope with a camera	√	√
Analysis of very high resolution images of regular wood cores or disks, or of woody roots acquired with a scanner or a camera	√	√
Image edition and debris filtering	√	√
Non cell objects and debris automatically filtered out by color		√
Cells can be grouped to be analysed as a single one (ex: several parts of a vessel)	√	√
Batch processing analysis mode	√	√

Visualize and Analyze morphological measurements with XLCell

XLCell is a utility program that runs in Microsoft Excel which purposes is to reclassify and visualize data produced by **WinCELL**. It can separate measurements data into different sheets as a function of their type (global, individual cells, interactive paths, annual ring) for one or many images.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	SampleId	PATH	Year	Image#	PathName	CellRank	Cell#	LumenArea	LumenLength	Wall1Length	Wall2Length	AvgWallLength	CellLength	Av
2	s10	PATH	2011	1	P1	1	47	83.2227	8.7712	0.5297	0.5297	0.5297	9.8305	
3	s10	PATH	2011	1	P1	2	58	89.5474	9.5763	0.5297	0.4873	0.5085	10.5932	
4	s10	PATH	2011	1	P1	3	65	95.6944	9.322	0.5085	0.4873	0.4979	10.3178	
5	s10	PATH	2011	1	P1	4	73	59.0955	6.1441	0.5085	0.4873	0.4979	7.1398	
6	s10	PATH	2011	1	P1	5	82	57.8985	6.1864	0.4661	0.5508	0.5085	7.2034	
7	s10	PATH	2011	1	P2	1	51	92.947	9.0678	0.4661	0.4661	0.4661	10	
8	s10	PATH	2011	1	P2	2	59	64.0748	6.7373	0.4661	0.4449	0.4555	7.6483	
9	s10	PATH	2011	1	P2	3	62	57.9024	6.6525	0.4449	0.5297	0.4873	7.6271	
10	s10	PATH	2011	1	P2	4	68	105.3952	10.7627	0.5297	0.5932	0.5614	11.8856	
11	s10	PATH	2011	1	P2	5	80	118.236	11.6949	0.5932	0.678	0.6356	12.9661	
12	s11	PATH	2011	2	P3	1	6	94.265	9.2373	0.6144	0.6356	0.625	10.4873	

Testpaths / Global / Cell / Group / **Path** / Ring

XLCell can merge sample data that has been analyzed in more than one image such as when a ring is too large or the camera resolution too low to be digitized in a single image. A command is provided to merge the measurement data of an annual ring split into two or more images as if it was acquired as one.

XLCell can display graphically different measurements for visualization or validation.
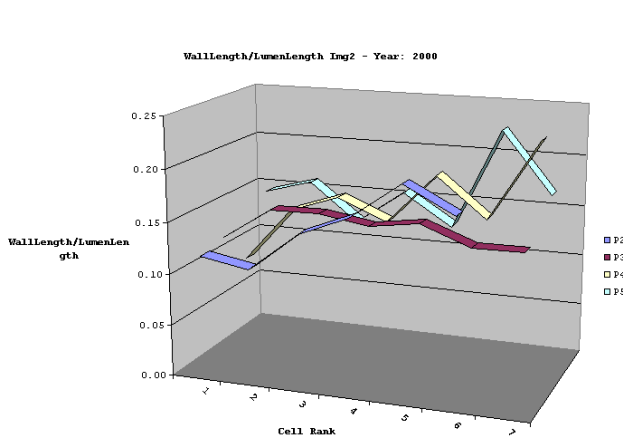
Wall Length to Lumen Length ratio for cells of four paths
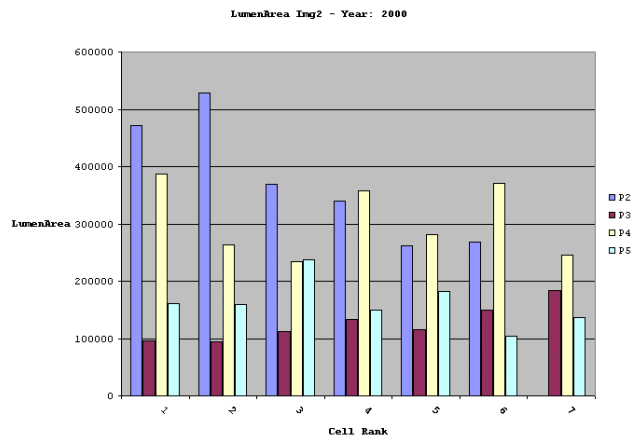
Lumen area for cells of four paths

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