

Drawing the short straw

Standard Operating Procedure (SOP) for the CoreProcessor (formerly known as Densitometry), RingIndicator (formerly known as DHXCT) and CoreComparison toolbox

We are improving the software so please consult the UGent-Woodlab team if you need more information. See also www.dendrochronomics.ugent.be for the latest version of the toolboxes.

When using these toolboxes, please refer to the following publications:

- Yan den Bulcke, J., Boone, M.A., Dhaene, J., Van Loo, D., Van Hoorebeke, L., Boone, M.N., Wyffels, F., Beeckman, H., Van Acker, J. and De Mil, T., 2019. Advanced X-ray CT scanning can boost tree ring research for earth system sciences. Annals of botany, 124(5), pp.837-847.
- De Mil, T., Vannoppen, A., <u>Beeckman</u>, H., <u>Van Acker</u>, J. and <u>Van den Bulcke</u>, J., 2016. A field-to-desktop toolchain for X-ray CT densitometry enables tree ring analysis. *Annals of botany*, 117(7), pp.1187-1196.
- 3. Van den Bulcke, J., Wernersson, E.L., Dierick, M., Van Loo, D., Masschaele, B., Brabant, L., Boone, M.N., Van Hoorebeke, L., Haneca, K., Brun, A., Hendriks, C.L.L., and Van Acker, J., 2014. 3D tree-ring analysis using helical X-ray tomography. *Dendrochronologia*, 32(1), pp.39-46.
- De Ridder, M., <u>Van den Bulcke</u>, J., Vansteenkiste, D., Van Loo, D., Dierick, M., Masschaele, B., De Witte, Y., Mannes, D., Lehmann, E., <u>Beeckman</u>, H., Van Hoorebeke, L., <u>Van Acker</u>, J. 2011. High-resolution proxies for wood density variations in *Terminalia superba. Annals of botany*, 107(2), pp.293-302.

Warranty disclaimer

It is not allowed to distribute these toolboxes without permission. The content of this software is based on the authors' experiences and is under continual revision. It is not intended as a standard. This software is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.' It can not be used for commercial purposes.

In any case, for all steps discussed in this manual, it is advised to try to check the results in a software package such as ImageJ. Some steps are automated and involve morphological operations, but it is the user's responsibility to check the validity of the results!



Sampling wood cores

We have a SOP for taking increment cores at UGent-Woodlab, upon request. The toolchain has been designed for cores that are stored in paper straws. However, the toolchain also works for unmounted cores and even sanded cores.

Preparing your paper straws¹

Make sure your entire paper straw is filled with wood. Every void is a loss of scanner volume so be efficient. Wrap both straw-ends up and cut the ends (Fig. 1). This enables easy insertion of the straws in the cylindrical holder. Make sure the cambium (bark) side is clearly indicated on the core. Bits and pieces are difficult to work with, as well as cracked cores. Given the 3D nature of all operations, it is better to have intact cores. Especially wedge-shaped split cores should not be put together for several reasons. Therefore, it is not advised to split the cores in such a way, or at least separate the parts while scanning to avoid processing issues afterwards. It is much easier to work with clean cuts / fractures within a ring or at a ring boundary, which can be corrected for. See further.

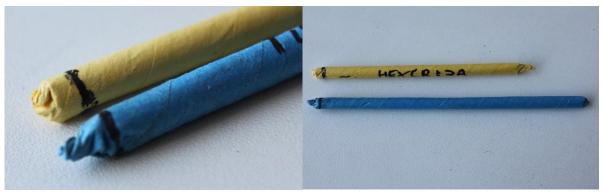


Fig. 1 Make a tight seal of the straw ends, note the indication of the cambium side

Choosing the cylinder type

Count your cores and measure the length. Based on this information you can choose among different cylinder types that we use at UGent-Woodlab: cardboard sample holder type 1 (holds 33 cores of any length) and sample holder 2 (holds 6 cores of 1-15cm length, has a 3cm diameter) for higher resolution scans or. These are examples, of course, and other types of sample holders can be used as well. The difference in holder type is important since it is related to the processing, especially for the conversion from attenuation coefficient to density (kg/m³). More information will be given further in the SOP.

Mounting the samples and filling in the Excel file

All paper straws should be mounted according to the Cylinder Template (see example 'template.pdf' for the sample holder type 1). Always write your sample number next to the number on your Cylinder Template document (ranging from 1 to 33 for the sample holder type 1), while simultaneously completing the provided Excel file (see 'XrayForm.Excel' for an example of how the Excel file looks like). The Excel file is important because it will be used when extracting the separate increment cores from the scanned volume.

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¹ Paper straws (artstraws) 6mm are sold at Marsival Duboccage (Belgium) or Heutink (same company in the Netherlands, check its availability in your country): https://www.marsival.be/artikel/alles-van-marsival/300_036216 / www.slimmerspelen.nl



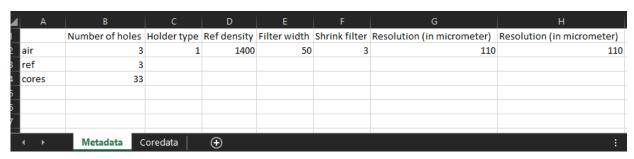
All cores should be inserted with the bark side down to avoid confusion, but this is not a requirement to run the software.

For multiple cores per hole, indicate the number of cores per hole; the sample label of the uppermost core is put in the first "sample" column! The additional cores should be filled in the consecutive columns (from top to bottom), if you put in more than three cores, you can add columns and fill in the label.

Avoid confusion and label your cylinder, give the Excel file the same name of the cylinder, and the template as well. Save each template in a separate Excel file.

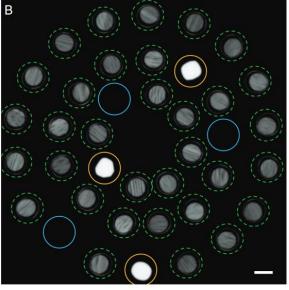
Afterwards, the other data of the Excel should be completed as well (diameter, location) and sent to the UGent-Woodlab team if you wish to collaborate with us.

Metadata page of the Excel file



You'll have to mention the number of holes with reference material, the number of holes with air and the total number of cores. This number should equal the number filled in on the 'CoreData' tab. The holder type is either 1 or 2, and as mentioned above this impacts the processing. An example given in the Figure below clarifies this difference.

Type 1



Type 2

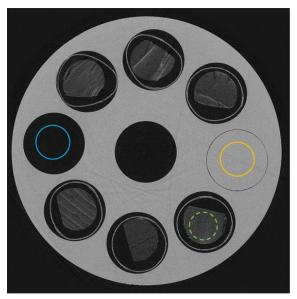


Fig. 2 (Left) Sample holder type 1, with reference material (encircled yellow for reference material and encircled blue for air) and increment cores (encircled green). Once selected, these increment core volumes contain paper, straw, air and actual wood. Therefore, some automated morphological operations are needed to isolate the wood. For more details, we refer to the publications mentioned above.



In the example below, you see the structure of the 'CoreData' tab. The first column should remain a numbered list, as long as the number of cores. The second column tells how many cores are in a single hole (although possible, not generally recommended because more cumbersome to work with), and their respective labels in the next columns. It is recommended to add as much metadata as possible for future reference / archiving purposes. Important: use letters only or letters and numbers in core labels, not only numbers!





Before using the software

Distribution of the software is not allowed without permission of the Laboratory of Wood Technology (Ghent University; UGent-Woodlab).

Make sure the correct MCR is installed on your computer, freely available on: http://nl.mathworks.com/products/compiler/mcr/ or ask at UGent-Woodlab. The version needed is mentioned on the dendrochronomics (www.dendrochronomics.ugent.be) website.

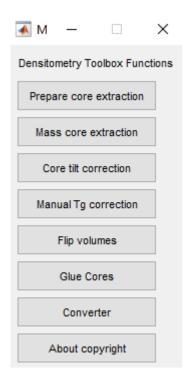
Make sure the Excel-file is properly completed and all metadata are filled in correctly.

Next to the folder of the reconstructed volumes, please make a new empty folder for the matfiles: e.g. "matfiles_useful_name_sample_holder", this is necessary to store temporary parameters.



CoreProcessor ToolBox

Below, the different options of the Densitometry Toolbox are given:



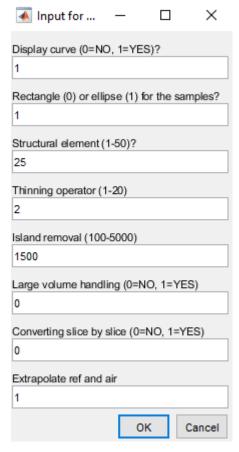
- 1. Prepare core extraction: this is to prepare the extraction of the increment cores (see Fig. 2). It is separated from 'Mass core extraction', for actual execution of the extraction, to allow for efficient processing
- 2. Core tilt correction: it is possible that the core is not perfectly straight positioned in the sample holder (only if working with soft materials like foam, will not be an issue for the plastic type 2 we use for e.g. for MXD measurements). This step will automatically find the optimal alignment. Can be skipped
- **3. Manual Tg correction**: to make sure that the transversal and radial plane are well-oriented, similar to what you would do when mounting physical samples in a sample holder before sanding: you want to visualize the transversal plane!
- **4. Flip volumes**: in case you need to flip cores, if the pith-bark direction is not left to right. i.e. in case you did not put the bark side down.
- 5. Glue cores: stitch parts of individual cores together
- 6. Converter: if you have been playing around with the volumes in another software package and have saved them outside the toolbox (e.g. ImageJ), this step is needed to get the header info correct. Interesting if you have 16-bit multipage tiff volumes coming from elsewhere and you want to use RingIndicator



In this step the user will indicate all the cores that are going to be extracted from the cylinder volume

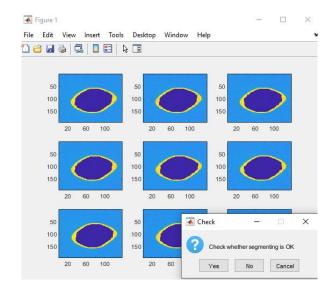
- Select the folder with reconstructed files (16-bit cross-sectional slices) and your Excel-file (XrayForm)
- The following screen appears





- 1. **Display curve**: will show you the greyscale profiles of air and refence material. Good to keep on '1' to make sure that these profiles look OK.
- 2. **Rectangle / ellipse**: you have the option to go for a rectangular or ellipsoidal indication of the cores / ref / air
- 3. **Structuring element / thinning operator / island removal**: all refer to morphological operators to result in the most optimal result for the extraction of the ref material profile, but used for sample holder type only. Leave the default values and change when the greyscale profile doesn't look good. Important step, better always check by calculating yourself for a couple of values (see **De Ridder et al. 2011** for the formula).
- 4. **Large volume handling**: if '1', then the processing will use less RAM but will take much longer. Only do so if insufficient RAM. Hasn't been error checked thoroughly
- 5. **Converting slice by slice**: if previous one is set to '1', also do so here, unless you can work on a high RAM PC for this step. Again, much slower (like in point 4).
- 6. **Extrapolate**: will give you the option to extrapolate the ref material average outside the ref material area. Same will be done for air based on ref indications. See further
- Select your newly created empty "mat file" folder or, if not created, select any other folder
- The volume is loaded and a longitudinal cross-section of the cylinder appears where users can select a few horizontal slices to check if all cores and references were correctly mounted into the cylinder. Click as much as you want from bottom to top to evaluate this. These crosssections will be used for the next step, so click across the entire length! Cores might not be running straight depending on the mounting.
- Then indicate the dark (air) and white references (Fig. 2), as many times as indicated in the Excel file. The average will be used for converting. Double click after each selection.
- For each ref material, you will see a Figure with 9 cross-sections through the ref material core. This way you can check of the morphological operations are correctly performed (the area used to calculate the average grey-scale per cross-section is indicated in purple, see example below) which is essential for correct conversion to densities. Just click any of the 3 buttons (Yes, No, Cancel) to move further. There are no functions behind those options.





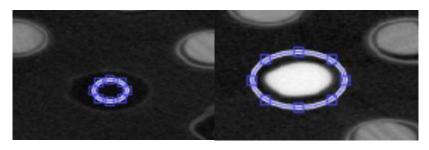
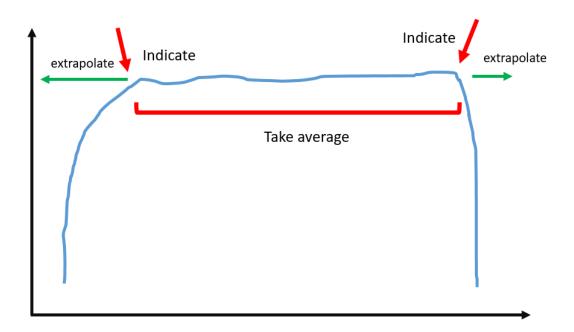


Fig. 3 (Left) Dark reference: draw an ellips that contains only air, (Right) White reference: draw an ellipse that reaches little beyond the boundaries of the reference material, an algorithm will auto-crop further. This is an example for sample holder type '1', for type '2' you'll indicate within the reference material as well.

Next, if **Extrapolate = 1**, a plot appears of the average greyscale values for the reference material. This has been implemented because in some cases your reference material might not be covering your entire sample lengthwise (e.g. shorter ref material), although you should avoid that and should also realize that the conversion might then differ slightly due to this extrapolation (for sample holder 2, as given in the figure, this extrapolation can't be done because outside the ref holder the X-ray attenuation will differ significantly and thus erroneous values are going to be the result). Since conversion from greyscale to density is done at each position, to consider vertical variation (from detector, tube, recon), this can't be done for areas at top or bottom where no reference material is available. Therefore, the average value (in between your 2 indications) is used to fill all positions before and after your indications. Make sure to check that this is OK afterwards. See Figure below for a schematic presentation of the approach. Again, for sample holder 2 it is advised to set this to 0! In any case, always ensure that your ref material covers the entire length of your samples and more, such that extrapolation is not needed.







A screen then appears where you have to select all the cores separately, please hold your Cylinder template with you and check if the autofill box is correct! Start from the correct position (Number 1, between the air and white reference on the outermost core belt as indicated on your Xray form / Cylinder template or any other sample holder that you might have designed with proper order / numbering system) and continue following the numbers. *Make sure you leave some space around the core to make sure the core is completely extracted.*

Note: In case of a very tilted sample holder or very titled cores, use software to Rotate the entire volume. At UGent-Woodlab, we use the in-house developed Octopus Analysis software (Volume loaded \rightarrow Tools Rotation (X,Y) \rightarrow Tools Scripting \rightarrow Export Z (as TIFF)). You can use ImageJ to do so as well, but don't forget to run all these manipulated cores to the Converter again.

This 'Prepare Core Extraction' step can be repeated for many different sample holders. Mass core extraction can then be executed at once for all of them, given that the metadata and Excel files are located in the same folder.



In this step, all the cores will be extracted according to the indications of the user (that are stored in the matfile folder). After the extraction, a new folder "Extracted" will be created which contains all the separate cores. When you have prepared multiple sample holders in the first step, and all the mat files are stored in the same folder, all mat-files in this folder will be processed. We have separated the prepare step from the actual extraction step, because in some case extracting all the cores and converting them to actual density values, can take a long time.

3) Core tilt correction



This step will automatically correct for core tilt (e.g. when the core is slightly inclinated in the cylinder). This is important for later steps. You just need to select the 'Extracted cores' folder created in the previous step, and this will be done automatically. **This is normally not needed if you have a sample holder type '2'.**

4) Manual Tg correction

In this step, all cores will be rotated along their length axis to the transversal direction (necessary for tree ring analysis, e.g. sanding or microtoming of wood surfaces on the transversal plane). Some cross-sections are displayed so that the user can see the grain direction. In the last plot (bottom right) a slice is presented where you can draw a line to indicate the grain direction. Double click, and the core will be automatically flipped. Then a screen appears where you can crop the volume of the core, make sure your rectangle encompasses the entire core diameter (Fig. 3). This is necessary because the central point of the rectangle should fall together with the core axis (for correct alignment in the RingIndicator module). If scanning at high resolution, you can also opt to select only

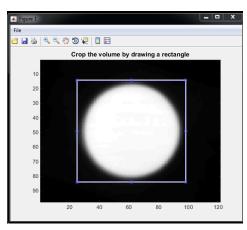


Fig. 4 Cropping of the volume

material within the core at this stage but then you should use the converter to switch to sample holder type 2. Could be helpful for ring-porous species for example (see further).

Now all steps are taken to perform structure direction corrections and finally, densitometry and tree ring analysis. *A minimum requirement of the basic principles of dendrochronology is preferred (see literature).*



RingIndicator toolbox

When you open the toolbox, you will be asked to open a multipage tiff file (one that was created after extraction, tilt correction or Tg correction; preferentially the latter, of course). After that, the 'Half thickness of slice image' allows you to display an average image of the central X slices, with X ranging from 1-10: 1 = no averaging, and only the central slices are shown, 10 = average image of the 10 central slices is shown. Averaging can be beneficial to highlight ring borders better, and suppress noise. Important: this is merely for displaying, it doesn't impact the underlying volume neither impacts the eventual calculations / results.

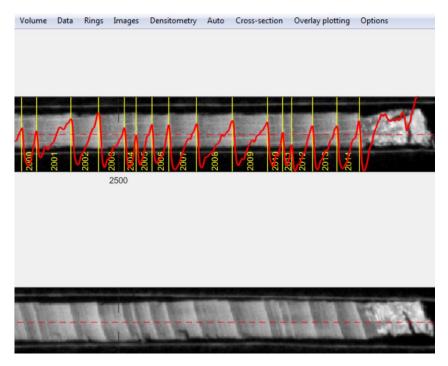


Fig. 5 Screenshot of a core opened in the RingIndicator toolbox

Via Volume → Load Volume and select the multipage Tiff file in the directory, these are located in the newly created **Tg corrected** folder or on any location you have stored them.

A Graphical User Interface appears with:

- Top figure: Transversal plane of the core
- Bottom figure: Radial plane of the core

The <u>very first step</u> to perform is a structure correction, using two options, but in both cases, one should indicate the start and end of your region of interest, in most cases the pith and last ring of the core. Tip: make sure to indicate the ring and fibre angle properly, because all subsequent indications will take an interpolated angle between respective indications:

- a) Manually click on the image to insert green bars for structure correction. Start with the pith and end with the most recent ring. Along the core you can correct for structural deviations on both the transversal and the radial plane. When a green bar is inserted on one plane, it will automatically generate one on the other plane. After the insertion, the nodes on the end of the bar can be moved to change the angle. The middle node can be used to change the bar position.
- b) Optional:



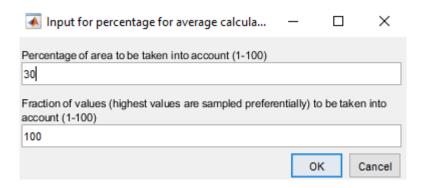
- a. Use the Auto → Suggest Angles, where the Structure Direction Algorithm will determine the structure (mainly useful for conifers, but you can always try). Leave all parameters default.
- b. If the result is not good (too many wrong angles), you can reset everything, or delete the rings with the large structure angles automatically (Data >> Remove largest angles) or manually by clicking at it with the right mouse button.

After every step, make sure you perform Data > Export, AND Rings > Export Rings, to be sure that you write the ring and fibre indications to the proper txt files. Throughout all corrections, make sure you always perform these two actions.

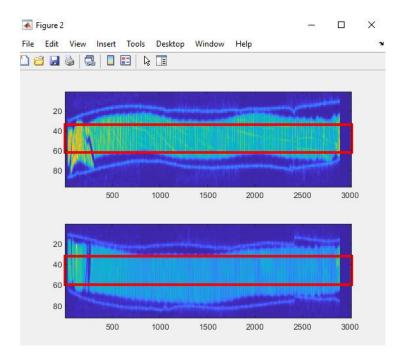
Second step is to create a rectified densitometry profile: press "Densitometry"

A densitometry profile is created and plotted, that can serve as an overlay for your core planes (Overlay plotting >> plot density profile). For ring-porous species such as oak, it might be necessary to finetune parameters for density profiling (15-1-500 instead of 25-2-2500) in case you work with the sample holder type 1 kind of processing, because then the morphological operations might remove the larger earlywood vessels and created and un-natural drop in density and the ring borders. In any case, switch on the fill holes and convexhull option for ring-porous species, it could largely solve the issue and includes vessels then to a large extent. If, however, you have many smaller cracks in your cores, and if not ring-porous, you might opt not to switch on this option to leave out the cracks. It is either one of both.

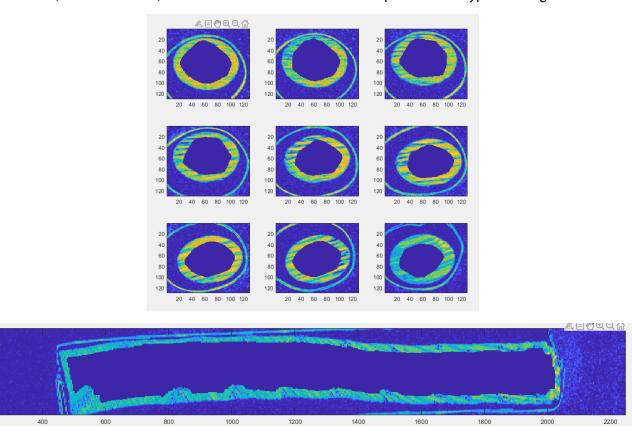
This will be resolution dependent as well. For high resolution scanning (e.g. 40 micron or below) of 5mm increment cores, it is recommended, if possible, to use sample holder type 2, where no morphological operations are needed to remove air or straw boundaries for density calculations and thus vessels are always included. You could always switch to sample holder type 2 after Manual Tg correction (see above). For sample holder type 2, you also get the option to select only part of the volume (percentage area considered) and red rectangles will show you the area. With this option, you can actually still have air surrounding the sample but have the red rectangles only covering the wood. The second option (fraction of values) then asks if you want the average of all pixels of that plane or only a percentage (5% = only 5 % highest are included = interesting option for MXD, to exclude lower values from the year after, resin ducts, etc ... for mean density values one should use 100%).







Below you can find the screenshots of the result on a ring-porous species scanned at 50 micron. The centrally located purple-covered zone is where the density values are taken. You can see that although much better with the convexhull approach, it is still not optimal at ring-boundaries. Therefore, in such a case, it could be useful to switch to sample holder 2 type after Tg correction.



Third step consists of two options again (cfr first step), that can be used in combination.

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- a) Manual indication of ring boundaries: as in step 1, a green bar can be inserted, this time the user can do this ring per ring. For visual crossdating with other cores from the same tree, open another RingIndicator application (by clicking on the icon again) and you can visually assess both cores on your screen (it is advisable that you use two monitors for this approach). Indeed, you can open as many instances from the toolboxes as you need.
- b) Auto Max/min/inflection detection. This function will automatically indicate tree ring boundaries based on the densitometry profile. Depending on the species (diffuse-porous/ring-porous), the maximum/minimum, or inflection can be used as a tree ring boundary criterion. The first parameter is a threshold for the peak function, the second is a SMOOTHER (Default 10), this value needs to be changed to 0!
- c) After you have performed the quasi-complete ring demarcation, please perform automatic shifting (with window size 1 and user-defined iterations, but make sure that no ring indications are plotted on each other due to this shift and reirun the densitometry plot. Then no adjustment should be made, and press data > export and ring > Export ring widths.

Change the felling date to the year at which the increment cores were taken on living trees or any other date that is suitable, and after the tree ring measurements, always export the data and the rings. In Overlay plotting, you can select "plot rings" and the years are displayed. You can also select the plane on which to plot rings and / or the density profile are displayed. After pairwise comparisons of cores are made, you can always import the rings, change them, and export them again. This iterative approach should lead you to a successful cross-dating and the creation of densitometry profiles.

The 'Rings -> check rings' option: this will check if you have indications that have a position outside the images and should this be corrected for, as well as rings that are overlapping, e.g. in case you double clicked twice on the same position. Check the numbers written in blue above the rings to spot them if there are such values. E.g. in the example below, you can see a '1' and then a '2'. The '2' is correct, but the '1' indicates that two indications are on top of each over. However, this only works if they are exactly at the same positions. It is possible that you want to have very narrow rings hardly visible, but that you still want to indicate, which is possible in this way.

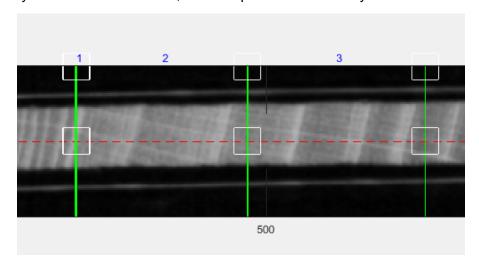
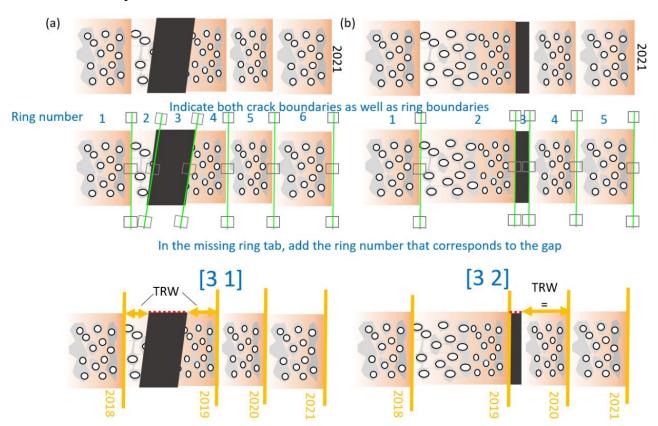


Fig. 6 Using the white boxes to move (middle one) or rotate (top and bottom one) the green indication lines

For broken cores that have bits and pieces: start at the pith, and demarcate anomalies (air due to broken cores, bright spots due to pockets, contamination and rotten areas) as if they were rings so you can delete them afterwards. End with the tree ring boundary of the last formed ring and export the data.



→ Both missing as well as broken rings can be indicated: see figure below. [missing rings to be mentioned]



By clicking on Images > Export interpolated slices, you can save the corrected volume, i.e. corrected according to the ring and fibre angle. This will completely skew the imagery, it could be helpful to display this to check whether ring and fibre angles have been corrected properly.

Several txt files are created, and the most important ones are:

- XXX_density.txt: uncorrected density values for the entire length of the image, thus not corrected for ring and fibre angle
- XXX density corr.txt: cf. previous txt but then with corrected density values
- XXX_ring-and-fibre.txt: ring and fibre indication positions and angles
- XXX_zpos.txt: position of the tree ring boundaries on your core, without ring and fibre correction
- XXX zpos.corr.txt: idem as zpos, but then corrected for ring and fibre angle
- XXX_ringwidth.txt: calculated ring widths, considering ring and fibre angle

If you want to calculate your own density values (for instance LW vs EW, or other metrics not supported by the CoreComparison and CoreProcessor Toolbox), you can make use of the zpos_corr and density_corr text files and use these for your own calculations. Of course, then you'll also have to work around any broken or missing rings indicated in the software, which will be recorded in the ringwidth.txt file (fifth and sixth column, among others).



Ring widths can be exported to commercially available dendrochronology tools (TsapWin, etc.) using the CoreComparison Toolbox.

Note: flatbed images

Next to X-ray volumes, flatbed images can be loaded into the RingIndicator module. Before importing these flatbed images, it is necessary to press the button of the Densitometry Toolbox. Then open the flatbed images in RingIndicator. All previous tools can be applied to flatbed images (except the fibre correction). These flatbed images can be linked to the X-ray volumes, through opening the latter in a new RingIndicator window, then Data Import from RingIndicator. A

reference point (e.g. a clear ring, or the cambial zone) needs to be chosen, in order to link these two images. This way you can have both visual (flatbed) as density information (XCT) to analyse the tree ring boundaries.

Note: When you manipulate your X-ray volumes in other software (e.g. when you cut volumes in ImageJ), always use the Converter to reconfigure your X-ray volumes!



CoreComparison toolbox

When using this toolbox, two windows are opened: the CoreComparison and the PatternMatching module.

This latter interface is analogous to TsapWin or any other tree ring analysis software. Not all the functions of the commercially available software are implemented here, because *it is by no means a complete analysis software to build chronologies*. The main goal is to have an idea of synchronicity of your ring width series, with a coupling to RingIndicator, without converting to ring width indices. This can be performed in a later stage with conventional software.

When you have completed the RingIndicator section, all ring files (*.txt) are displayed, select the text files you want to compare, and a screen will open with ring widths, together with Glk (i.e. ppr) and correlation parameters (Fig. 5). If you want to make changes to these ring width profiles, re-open RingIndicator, import data, export the modified data and rings, and use the refresh button in the Core Comparison module (see top left-hand side) to see the changes. You can open multiple RingIndicator instances to make changes to different tree-ring series.

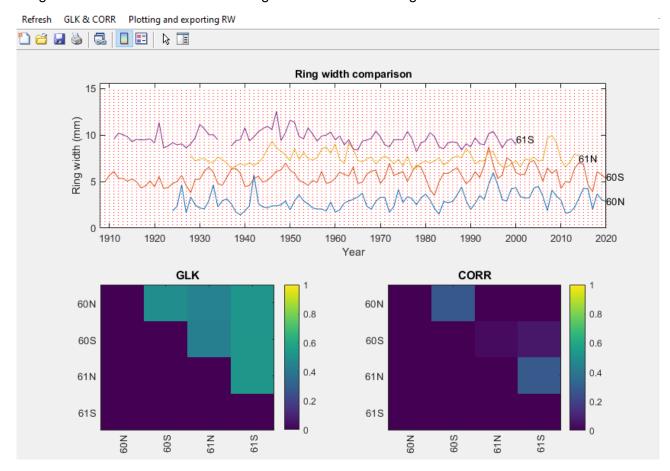


Figure 2: Screenshot of the Core Comparison module, note that the ring width series are shifted for easy visual comparison

In the GLK & CORR menu, a series of action scan be performed related to GLK, such as thresholding the figures, exporting the GLK values to Excel and ranking them.

The Plotting and Exporting function allows you to change the offset between the curves (a larger value results in a smaller offset), visualize the RW data in a plot (Plotting and Exporting→ Ring width) and finally, the RW data can be exported (Plotting and Exporting→ Export RW data).



The PatternMatching toolbox contains a series of functions to play around with the density data. It is the toolbox used to export the average density value per tree ring, the MXD value and the density per quartile of every tree ring. Make sure that all the indications are within the span of the densitometry profile, and that no indications are plotted on each other, because these indications will give erroneous results.

The first thing to do is to run Loading, running and ranking → load and plot pattern-matching data.

The result is shown in Figure 6.

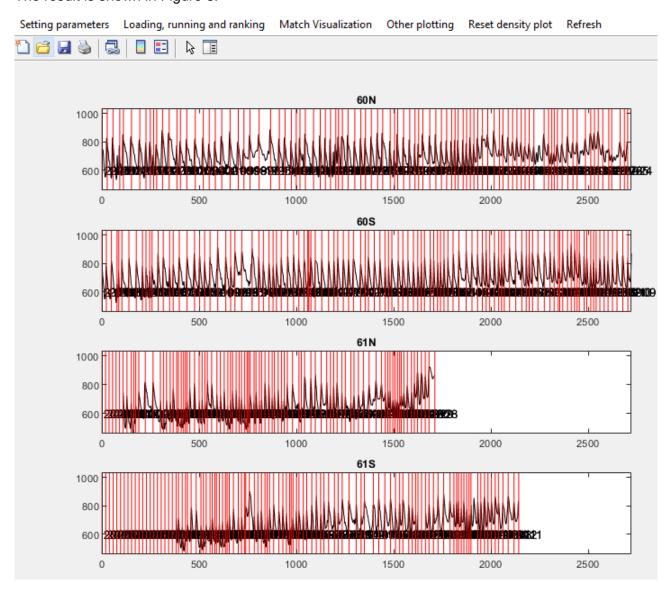


Figure 6: Screenshot of the PatternMatching module. Each core has its own density profile and ring indications.

Then, Loading, running and ranking → run pattern-matching using the standard settings.

Depending on the number of cores selected, this calculation can take a while. The amount of RAM on your computer, will also determine the number of cores you can load (the software will crash or will give an error message in case too many cores have been loaded at the same time).



When you are only interested in the density profile and want to play around with the data yourself, use the txt files from the DHXT toolbox (see above).

For obtaining the average density, MXD, MND and quartile data per tree ring, use Other plotting > Export clustered data. The tabs in Excel file you get is detailed below.



- Mean density: mean density per tree ring
- Max density half-based: MXD but only based on the second half of the tree ring to avoid that high density values of the previous year are not included
- Min density: the minimum density per tree ring
- Q1-Q4: mean and max for each quartile of every ring

Additional remarks

If users want to obtain the mean density of the total core, they should average the entire profile and **NOT average all the mean ring densities**, since this would assume that all rings have the same ring width.

After using the software

Please note that the obtained profiles are <u>estimates</u> of specific gravity, being dry weight/dry volume, or when conditioned at a certain RH [weight at u%]/[volume at u%]. The observed trends are correctly presented, but if you want to have <u>exact density values (e.g. for carbon quantification)</u>, a calibration with the water immersion (or geometric) method for a subset of your samples (per species) is necessary to further fine-tune the specific gravity estimates. This can be done at our lab, according to a standardised set-up.