

1 **TITLE:**

2 Tree Core Analysis with X-ray Computed Tomography

3

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19 **SUMMARY:**

20 Here we show how to process tree cores with an X-ray computed tomography toolchain. Except
21 for chemical extraction for some purposes, no further physical lab treatment is needed. The
22 toolchain can be used for biomass estimations, for obtaining MXD/tree-ring width data as well
23 as for obtaining quantitative wood anatomy data.

24

25 **ABSTRACT:**

26 An X-ray computed tomography (CT) toolchain is presented to obtain tree-ring width (TRW),
27 maximum latewood density (MXD), other density parameters, and quantitative wood anatomy
28 (QWA) data without the need for labor-intensive surface treatment or any physical sample
29 preparation. The focus here is on increment cores and scanning procedures at resolutions ranging
30 from 60 µm down to 4 µm. Three scales are defined at which wood should be looked at: (i) inter-
31 ring scale, (ii) ring scale, i.e., tree-ring analysis and densitometry scale, as well as (iii) anatomical
32 scale, the latter approaching the conventional thin-section quality. Custom-designed sample
33 holders for each of these scales enable high-throughput scanning of multiple increment cores. A
34 series of software routines were specifically developed to efficiently treat three-dimensional X-
35 ray CT images of the tree cores for TRW and densitometry. This work briefly explains the basic
36 principles of CT, which are needed for a proper understanding of the protocol. The protocol is
37 presented for some known species that are commonly used in dendrochronology. The
38 combination of rough density estimates, TRW and MXD data, as well as quantitative anatomy
39 data, allows us to broaden and deepen current analyses for climate reconstructions or tree
40 response, as well as further develop the field of dendroecology/climatology and archeology.

41

42 **INTRODUCTION:**

43 Wood density is an easy-to-measure variable¹ that reflects both the anatomical and chemical
44 properties of the wood². In biomass estimations of aboveground biomass, wood density is an

45 important weighing variable³⁻⁵, that is multiplied with the dimensions of the tree and a factor
46 representing the carbon content of the wood. Wood density is tightly linked to the mechanical
47 properties of timber⁶ and reflects the life history of a tree⁷.

48
49 Cell wall density is measured as being approximately 1500 kg/m³ and is considered fairly
50 constant⁸, however intra-ring cell wall density variations should be considered as well^{8,9}. Woody
51 cells (in general tracheids in conifers, vessels, parenchyma and fibers in hardwoods) are
52 oriented/shaped in different ways and cell wall thickness and lumen size of these cells varies¹⁰.
53 Therefore, wood density varies between trees, within a tree (axial and transversal) and within
54 short intervals within a tree ring^{11,12}. In many cases the wood density variation at the ring scale
55 also delimits the tree ring boundary¹³. Wood density and ultimately tissue fractions are
56 generated and in this paper are broadly put into three categories (i.e., three different resolution
57 scales), depending on the study goal (**Figure 1**) as described below.

58
59 **Inter-ring scale:** By measuring pieces of wood, a single value is obtained for that sample. This can
60 be done through water immersion or geometrically¹⁴. This way, general biomass or wood
61 technological variables can be obtained. To include pith-to-bark variation, these pieces of wood
62 can be further divided into blocks that are measured manually to obtain information on the life
63 history strategy¹⁵. When switching to low-resolution X-ray CT such as in medical scanners^{17,18},
64 TRW data on medium-to-wide rings can be made in an efficient way on many samples¹⁸⁻²⁰. This
65 is also the scale that can be used to assess biomass from pith-to-bark from both temperate and
66 tropical trees^{4,22}, typically ranging in resolutions from 50 µm to 200 µm.

67
68 **Ring scale:** Wood is a recorder of past environmental conditions. The best known parameter is
69 tree-ring width (TRW), but for global temperature reconstructions, maximum latewood density
70 (MXD) records are proven to be a better proxy for temperature²². MXD is an easy-to-measure
71 variable²³, and a proxy for cell wall thickness and cell size on the last cells of a tree ring, and are
72 at tree line and boreal sites positively linked to seasonal air temperature²⁴: the warmer and
73 longer the summers, more cell wall lignification occurs which thus increases the density of these
74 last cells. Traditional measurements such as immersion and geometry are less accurate to
75 determine this ring-level density. A previous work developed a toolchain for using X-ray film on
76 thin-cut samples²⁵. This sparked a revolution in both forestry and later paleoclimatology^{15,18},
77 defining maximum latewood density (MXD), i.e., the peak density value often at the end of a ring,
78 as a proxy for summer temperature. The basic principle is that the samples are sawn
79 (approximately 1.2 mm to 7 mm¹³) to be perfectly parallel to the axial direction, and the sample
80 is put on a sensitive film exposed to an X-ray source. Then these radiography films are read out
81 through a light source that detects the intensity and saves the profiles and the annual tree ring
82 parameters. These tools, however, require a significant amount of sample preparation and
83 manual work. Recently this has been developed for X-ray CT in a more standardized way or based
84 on mounted cores²⁶. Resolution here ranges between 10 µm and 20 µm. TRW is measured on
85 this scale as well, especially when dealing with smaller rings.

86
87 **Anatomical scale:** At this scale (resolution < 4 µm), the average density levels become less
88 relevant as the main anatomical features are visualized and their width and proportions can be

89 measured. Typically, this is done through making microsections or high-resolution optical scans
90 or micro- μ CT scans. When the ultrastructure of the cell walls needs to be visualized, scanning
91 electron microscopy is the most commonly used method²⁷. At the anatomical scale, the individual
92 tissue fractions become visible so that physiological parameters can be derived from the images.
93 Based on the individual anatomical parameters and the cell wall density of wood, anatomical
94 density can be derived for comparison with conventional estimators of wood density²⁴.

95
96 Due to improved sectioning techniques and image software^{29,30}, dendro-anatomy³⁰ has been
97 developed to have a more accurate record of the wood, both to have a closer estimate of the
98 MXD in conifers and to measure several anatomical variables from broadleaf trees. On this scale,
99 actual anatomical parameters are measured and related to environmental parameters³¹. With
100 μ CT this level can be obtained as well^{32, 33}.

101
102 As wood is inherently hygroscopic and anisotropic, wood density needs to be carefully defined
103 and the measurement conditions need to be specified, either as oven-dry, conditioned (typically
104 at 12% moisture content) or green (as felled in the forest)³⁴. For large samples and technical
105 purposes, wood density is defined as the weight divided by its volume at given conditions.
106 However, the value of wood density is strongly dependent on the scale at which it is measured,
107 for instance from pith-to-bark wood density can double, and on a ring scale (in conifers) the
108 transition of earlywood to latewood results in a significant rise in wood density as well, with a
109 peak at the ring boundary.

110
111 Here, an X-ray CT scanning protocol of increment cores is presented in order to measure features
112 at the aforementioned 3 scales (**Figure 1**). Recent developments in X-ray CT can cover most of these
113 scales, due to a flexible set-up. The research goals will determine the eventual protocol for
114 scanning.

115
116 A crucial limiting factor (which is inherently connected to the scaled nature of wood density and
117 wood in general) is the resolution and time necessary for scanning. Examples demonstrate how
118 to: (i) obtain inter-ring tree scale wood density profiles for biomass estimations in *Terminalia*
119 *superba* from the Congo Basin, (ii) obtain density records from Clanwilliam cedar (*Widdringtonia*
120 *cedarbergensis*) based on helical scanning on a HECTOR system³⁵, and (iii) measure vessel
121 parameters on sessile oak, on the Nanowood system. B both scanners are part of the suite of
122 scanners at the UGent Center for X-ray Tomography (UGCT, www.ugct.ugent.be), a core facility
123 of UGent.

124
125 [Place **Figure 1** here]

126 **X-CT research in-on wood**

127
128 **Set-up of a scanner:** A standard X-ray CT scanner consists of an X-ray tube, an X-ray detector, a
129 rotation stage, and a set of motors to move the rotation stage, and in most cases also the
130 detector, back and forth (**Figure 2**).

131
132 [Place **Figure 2** here]

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cm,Gauche

133
134 Most lab-based systems have a cone-beam geometry, which means that the produced X-rays are
135 distributed from the tube's exit window in a cone-beam shape, implying that by changing the
136 distance between the object and the tube (SOD = Source-Object-Distance) and the detector and
137 the tube (SDD = Source-Detector-Distance), the magnification is controlled (see the discussion on
138 resolution). Due to the penetrating power of X-rays, they pass through the object, and the
139 intensity of the attenuation beam is a function of the energy of the X-ray beam, the chemical
140 composition of the object (the atomic number of the elements present) and the density of the
141 material. Given a ~~constant~~ energy ~~spectrum-constant~~ and a constant material composition of
142 wood, the attenuation of the X-ray beam is highly dependent on the density of the material,
143 which explains its use for densitometry. The attenuation (or transmission) can be expressed by
144 the Beer-Lambert law:

$$I_d = I_0 e^{-\mu d}$$

145
146 with I_0 the incoming X-ray beam exponentially which decays to a transmitted X-ray beam I_d when
147 propagating through the material over a distance d . The linear attenuation coefficient μ depends
148 on a series of interactions with the material of the object. The projections are thus recordings of
149 the transmitted beam.

150
151 Practically, the object is mounted on the rotation stage, a proper SOD and SDD are selected, a
152 certain power is selected as well (related to object size, density, and composition) and the object
153 is rotated 360° and during that rotation multiple projections are taken. These projections are
154 then used to reconstruct the interior structure of the object. There are several reconstruction
155 algorithms available, of which the most used ones are still based on the analytical framework
156 developed decades ago, relying on the Radon transformation and the Fourier slice theorem. For
157 more details, the reader is referred to specialized literature³⁶.

158
159 **Conundrum of resolution, data volume and sample size:** Resolution is key in X-ray CT scanning.
160 In systems with inverse geometry, or parallel beam geometry such as synchrotron beamlines,
161 other considerations play a role. This protocol only discusses standard lab-based X-ray CT
162 scanning with cone-beam geometry. Here, the concept of magnification, detector pixel size and
163 spot size are essential. Magnification is defined as the ratio of SDD/SOD. Next, the pixel size of
164 the detector obviously also impacts the resolution: the smaller the pixel size, the higher the
165 resolution, but in most cases the field of view (FoV) is also directly related to pixel size and size
166 of the detector (smaller pixel size, smaller FoV for the same number of pixels). Further, the spot
167 size of the X-ray beam is also important: the larger the spot size, the lower the resolution, which
168 means that less details can be seen.

169
170 It is important to address that one could obtain a resolution that is higher than what is possible
171 according to the abovementioned limits, therefore it is better to use the term voxel size (a voxel
172 is a volume pixel) instead of resolution. Moreover, there are other factors at play, such as
173 detector sharpness, which further limit the true resolution at which an object is scanned. Only a
174 true calibration of the system, using established targets, supplies the truthful answer.

175

176 In most cases, however, the voxel size at which an object can be scanned is mostly limited by the
177 size of the object. This means that the larger the object, the lower the voxel size will be. If the
178 object does not fit within the FoV of the detector for a certain voxel size, then the voxel size can
179 be reduced, e.g., by limiting the magnification.

180
181 Scan time and data volume are important to consider when deciding the desired voxel size. In
182 general, the smaller the voxel size means the higher the detail one wants to see, the smaller the
183 sample or the fewer samples that can be scanned at once, more time is required and larger the
184 data volumes will be collected. Imagine the following theoretical example: one can scan a sample
185 measuring 10 cm x 10 cm x 10 cm at 50 μm at once with a certain X-ray CT system and would like
186 to scan that same volume at 10 μm , the volume that fits within the FoV would only be 2 cm x 2
187 cm x 2 cm, assuming that this is physically possible. This means that 125 scans are needed ($5^3 =$
188 5 times higher resolution, scales to the power of 3 due to the volumetric nature of the imaging
189 technique) to cover the entire volume, and that the data volume would increase likewise. Of
190 course, this is only a thought experiment, and one needs to consider much more than only
191 resolution. For more information, the reader is referred to an overview of the scanning
192 possibilities³⁷.

193
194 **Flexibility of instruments for scanning of wooden objects:** In the last decade, many companies
195 have delivered X-ray CT systems with a similar assembly as HECTOR³⁵. An overview of several CT
196 systems, especially assessed for their temporal resolution, is given in³⁸.

197
198 In all, the flexibility and user-friendliness of X-ray CT systems has improved considerably. Many
199 systems allow scanning of a diverse range of objects, which is also the case with the systems at
200 UGCT. The protocol below is demonstrated for ~~the two of the available systems, HECTOR and~~
201 ~~the commercial CoreTom (a TESCAN XRE scanner) that are suited for tree core analysis system,~~
202 which is suitable for tree-ring analysis. The protocol however is valid for any other available
203 system if resolution and data format allows for it.

204
205 These systems allow the scanning of a variety of objects. A few pictures of different wooden
206 objects scanned with the HECTOR system are given in **Figure 3**. It is this flexibility that comprises
207 the three scales we present in **Figure 1**, ranging from a coarse resolution to a very fine resolution.

208
209 [Place **Figure 3** here]

210
211 **PROTOCOL:**

212
213 **1. Core sampling**

214
215 **1.1. Sample the tree with a Pressler borer.** Consult references on how to core a tree
216 manually³⁹. This toolchain is presented for 5.15 mm cores.

217

218 1.2. Put the tree cores unglued into 6 mm paper straws or in any other receptor that does not
219 require glue. Do not glue the tree cores on a wooden support. If the cores were already mounted,
220 unmount them with scalpel/saw or solvent depending on the glue type.

221
222 1.3. Avoid wrapping them in plastic tubes, try to dry them first to avoid mold growth and
223 fungal decay.

224 1.4. Use pencil to write on the paper straws, since the extraction could fade marker writings.

225
226 NOTE: This toolchain is also suited for 4 mm cores. Already mounted and sanded cores can be
227 used, but these need to be unmounted first. For archeological or remnant wood, a core-sized
228 sample needs to be made (by sawing/reducing the size) for scanning.

230 2. Core preparation before scanning

231
232 2.1. Perform an ethanol/toluene or ethanol or acetone⁴⁰ Soxhlet extraction for at least 24 h
233 (Figure 4A,B). This is necessary to ensure a resin-free wood volume to have an accurate density
234 calculation (resin attenuates the signal, Figure 4C^{13,41}).

235
236 [Place Figure 4 here]

237
238 2.1.1. Make a solution of 1000 mL of ethanol with 427 mL of toluene (according to e.g., ASTM
239 D 1107 – 96) or pure ethanol or acetone. Many universities have a lab glass workshop that can
240 make an adapted version of the Soxhlet apparatus for tree cores (Figure 4B).

241
242 2.1.2. Put-Fill the round-bottomed flask with the solvent, and put the samples within the straws
243 in a stainless-steel support in the Soxhlet apparatus (~~with Ethanol-toluene solution~~) with cooler
244 on a laboratory heater. Allow for at least 6 siphoning's per h.

245
246 NOTE: A closed water circuit is preferably used for cooling (or any other modern cooling system).
247 In this case, a water pump circulates the water, stored in a 100 L basin with chlorine tablets (to
248 avoid algae development), at 120 L/h (but this will depend on the size of the Soxhlet apparatus
249 as well as the applied heat by the heat element). An air cooler is used to cool the water in the
250 100 L basin. To save on solvent, glass beads can be used to fill-up the void volume of the Soxhlet
251 apparatus.

252
253 2.1.3. Check the temperature and number of siphoning's regularly. Dry the cores under a
254 chemical hood (check lab policy for this) to remove the solvent residue or put a drying oven under
255 the chemical hood.

256
257 2.2. Dry the cores for 24 h in a drying oven (103.5 °C) under exhaust ventilation and perform
258 a hot water bath (90 °C) or hot water Soxhlet extraction for 24 h as described above.

259
260 2.2.1. Keep the samples in the paper straws in the steel sample holders. Dry again in a dry oven
261 for 24 h at 103.5 °C, then condition to 60% relative humidity (RH) before scanning.

262
263 NOTE: The 60% value is chosen in this case because these are approximately the ambient average
264 relative humidity conditions in the X-ray CT scanning room. Oven dry samples can also be used.
265 The most important factor is that all samples are scanned under the same conditions.

266
267 2.2.2. In case the water extraction degenerates the paper straws, put the tree cores back into
268 new 6 mm paper straws²¹. Both 5.15 mm and 4 mm cores can be put into 6 mm paper straws.

269
270 2.2.3. Make sure the entire paper straw is filled with wood. Wrap both straw-ends up and cut
271 the ends. This enables easy insertion of the straws in the cylindrical holder.

272
273 2.2.4. Make sure the cambium (bark) side is clearly indicated on the core because the
274 CoreProcessor and RingIndicator assume that the bark side is down. Remove bits and pieces as
275 well as cores with cracks as these are difficult to work with.

276 277 **3. Core scanning**

278
279 3.1 Select the proper sample holder type (**Figure 5**) as described below, depending on the
280 research purpose as described in **Figure 1**.

281
282 3.1.1 For inter-ring scale purposes, use holder type1 – cardboard and perforated floral foam.

283
284 3.1.2 For the MXD data use holder type 2 – a plastic sample holder that holds 6 cores of 1–15
285 cm length and has a 3 cm diameter.

286
287 3.1.3 Use holder type 3 – for dendro-anatomy purposes that consists of simply tying paper
288 straws together.

289
290 [Place **Figure 5** here]

291
292 3.2 Load the tree cores in paper straws into the sample holder and fill in the spreadsheet
293 template that can be downloaded on <https://dendrochronomics.ugent.be/#software>. Each
294 number corresponds to a given position in the sample holder.

295
296 3.3 Scanning in an X-ray microCT- μ CT facility: consult an X-ray CT expert for the proper
297 settings and scanning protocol. For the scanner used in this protocol, see ³⁷.

298
299 3.4 Reconstruction of the scanned images: consult an X-ray CT expert for the proper settings
300 of the reconstruction, or the settings that come with the CT scanner (software package of the X-
301 ray CT scanner, ~~freely available~~). For reconstruction parameters for this protocol, see ³⁷.

302 303 **4. Obtain TRW data, density data and anatomical data**

304
305 4.1. Getting started with the software.

306
307 4.1.1. To obtain density values (holder type 1 and 2), install the following three software
308 packages: (i) CoreProcessor, (ii) RingIndicator and (iii) CoreComparison toolboxes from
309 www.dendrochronomics.ugent.be.

310
311 4.1.2. Since these toolboxes were made in MATLAB, install the correct MATLAB compiler
312 runtime (MCR), available on: <http://nl.mathworks.com/products/compiler/mcr/>. The version
313 needed currently is ~~MATLAB2021B~~[MATLAB2022B](http://nl.mathworks.com/products/compiler/mcr/). The MCR is free, just as the 3 software
314 packages.

315
316 4.1.3. Next to the folder of the reconstructed volumes, make a new empty folder for the MAT
317 files (a file format from MATLAB that stores variables, etc.), e.g.,
318 matfiles_useful_name_sample_holder, this is necessary to store temporary parameters.

319
320 4.1.4. Make sure the spreadsheet is in the folder of the reconstructed volumes, in order to fill
321 in the samples in a later step.

322
323 **4.2 Preprocessing the core volumes with the CoreProcessor.**

324
325 NOTE: For high-resolution anatomy data, following processing steps are not necessary (**Figure 1**),
326 once the reconstructed volumes are available, refer to the ImageJ guidelines for 3D images⁴².
327 Segmentation can be done through the Weka plugin⁴³ for indicating vessels on individual slices
328 for instance¹², or in software plugins such as ROXAS⁴⁴.

329
330 **4.2.1 Prepare the core extraction: Prepare the automated extraction of the increment cores**
331 **(depending on inter-ring scale **Figure 6A** or ring scale **Figure 6B**) as described below.**

332
333 **4.2.1.1 Select the folder with reconstructed files (16-bit cross-sectional TIFF slices) and the**
334 **spreadsheet file (XrayForm.xlsx).**

335
336 4.2.1.2 Select the empty folder from 4.1.3. This is the folder where CoreProcessor will create a
337 MAT file that will be used in 4.2.2. for mass core extraction.

338
339 4.2.1.3 Select the newly created empty MAT file folder or, if not created, select any other folder.
340 The volume is loaded, and a longitudinal cross-section of the cylinder appears, check a few
341 horizontal slices to ensure if all cores and references were correctly mounted into the cylinder.

342
343 **4.2.1.4 Click from bottom to top to select slices to evaluate this. These cross-sections will be used**
344 **for the next step, so click across the entire length. Indicate the dark (air) and white references**
345 **(**Figure 6**), as many times as indicated in the spreadsheet file. Double click after each selection.**

346
347 4.2.1.5 For sample holder 1, inspect the multiscreen with 9 cross-sections, generated through the
348 reference material to check whether the density calculation was performed on the wood (and

349 not on the paper straw or other non-wood material). This is essential for correct conversion to
350 actual wood density values.

351

352 [Place **Figure 6** here]

353

354 4.2.1.6 In a new pop-up window, select every core separately by drawing a circle/ellipse around
355 it. Perform labelling based on the information from the spreadsheet file, check whether the name
356 is correct. Respect the order in which the template is filled. All info is stored in a MAT file, that
357 will be used during the next step for extracting all cores at once.

358

359 4.2.2 Click on **Mass Core Extraction** to carry out core extraction for all the cores in a given
360 cylinder (that were indicated in 4.2.1.2), given that the metadata and spreadsheet files are
361 located in the same folder.

362

363 NOTE: When using Mass core extraction, all cores will be extracted according to the labelling of
364 the user (that are stored in the mat file folder). As a result of this operation, a new folder named
365 **extracted-Extracted** will be created which contains all the separate cores. When multiple sample
366 holders are prepared in the first step, and all the ~~mat~~-MAT files are stored in the same folder, all
367 ~~mat~~-MAT files in this folder will be processed.

368

369 4.2.3. In the ~~CoreComparison~~-**CoreProcessor** toolbox, click on **Manual Tg Correction**, then select
370 the **Extracted** folder to ensure proper orientation of the transversal and radial plane of every
371 core volume, similar to mounting physical samples in a sample holder before sanding keeping the
372 transversal plane in view (**Figure 7B**).

373

374 4.2.3.1. Some cross-sections are displayed so that the user can see the grain direction. In
375 the last plot (bottom right), inspect the slice that is presented and draw a line to indicate the
376 grain direction. Double click to automatically ~~flip~~-rotate the core.

377

378 4.2.3.2. Then a screen appears, here crop the volume of the core (**Figure 7C**), making sure
379 that the rectangle encompasses the entire core diameter. This is necessary because the central
380 point of the rectangle should be as close as possible to the core axis (for correct alignment in the
381 RingIndicator module).

382

383 [Place **Figure 7** here]

384

385 NOTE: If scanning at high resolution, select only material within the core at this stage and use the
386 converter to switch to sample holder type 2. This is certainly helpful for ring-porous species for
387 example.

388

389 4.2.4. Perform the following optional steps in case of misaligned, flipped, or broken cores or
390 incompatible image formats.

391

392 4.2.4.1. Core tilt correction (only for sample holder 1): To automatically correct for core
393 tilt (e.g., when the core is slightly inclined in the cylinder; **Figure 7A**). This is important for later
394 steps. Select the **Extracted Cores** folder created in the previous step.

395
396 NOTE: This is normally not needed if you have a sample holder type 2 or type 3 (**Figure 5**) or when
397 all samples and sample holders are put perfectly upright.

398
399 4.2.4.2. Flip volumes: In case the sample was not put with the bark side down (the pith-
400 bark direction is not left to right), change the direction by flipping cores. The function asks for a
401 folder and will create a new folder.

402
403 4.2.4.3. Glue cores: Stitch large parts of individual cores together virtually. The idea is not
404 to stitch perfectly, but to make sure that, if needed, single cores are in the same multipage TIFF
405 volume. Use the broken core indication (see step [54.3.14.109](#)) to overcome the spaces in
406 between the core pieces.

407
408 4.2.4.4. Converter: If the volumes have been manipulated in another software package
409 and are saved outside the toolbox (e.g., ImageJ), perform this step to get the header info correct.
410 Use this step for 16-bit multipage TIFF volumes coming from another CT scanner as well and for
411 using the RingIndicator toolbox.

412
413 NOTE: All steps from here are for structure direction corrections and finally, densitometry and
414 tree ring analysis.

415 416 4.3. Correcting the angles and indicating rings in RingIndicator.

417
418 NOTE: Following steps need to be taken chronologically in the RingIndicator module: (i)
419 visualization of the transversal and radial plane, (ii) structure correction, (iii) creating the density
420 profile, and (iv) indicating the rings manually or semi-automatically.

421 422 4.3.1. Perform visualization of transversal and radial plane as described below.

423
424 4.3.1.1. ~~Open As an introduction~~To get familiar with the GUI, open the toolbox and select
425 a multipage **TIFF File** (one that was created after extraction and ~~corrected after~~ tg corrected, so
426 from the folder name **Tg corrected**).

427
428 4.3.1.2. Select the **Half Thickness of Slice Image** to display an average image of the central
429 X slices, with X ranging from 1-10 with 1 being no averaging (i.e. only display the central slice),
430 and ~~only the central slices are shown, and~~ 10 being an average image of the 10 central slices ~~is~~
431 shown.

432
433 NOTE: Averaging can be beneficial to highlight ring borders better, and suppress noise, on the
434 other hand it can also make ring borders look less distinct, especially when the fiber angle is
435 substantial. Please tune this value according to the needs. It is important to mention that this is

a mis en forme : Police :Gras

436 merely for displaying, it does not impact the underlying volume, nor does it impact the eventual
437 calculations/results.

438
439 4.3.1.3. Inspect the graphical user interface (GUI). The GUI contains a top figure with the
440 transversal plane of the core and a bottom figure with the radial plane of the core, and a menu
441 (**Figure 8**).

442
443 [Place **Figure 8** here]

444
445 4.3.1.4. ~~Under the Volume tab~~To load a new core volume, search for the **Volume > Load**
446 **Volume** function to select ~~the a new~~ multipage **TIFF file** in the directory. Now the user is ready
447 to indicate the structure direction to subsequently indicate the actual tree rings.

448
449 4.3.2. **Structure correction to facilitate automatic tree ring indications.**

450
451 4.3.2.1. Manually click on the image to insert green bars for structure correction. Start
452 with the pith (or oldest ring available) and end with the most recent ring. Along the core correct
453 for structural deviations on both the transversal and the radial plane.

454
455 4.3.2.2. Make sure to indicate the ring and fiber angle properly, because all subsequent
456 indications will take an interpolated angle between the respective indications.

457
458 4.3.2.3. Insert a green bar on one plane, it will automatically generate one on the other
459 plane. After inserting the bar, move the nodes (white squares) at the end of the bar to change
460 the angle. Use the middle node to change the bar position. Adjust the size of the nodes for ease
461 of use.

462
463 4.3.2.4. After every step, make sure to press **Data > Export** and **Rings > Export Rings**, to
464 ensure that ring and fiber indications are written to the proper .txt files. Throughout all
465 corrections, make sure to always perform these two actions.

466
467 4.3.3. **Calculating the density profile.**

468
469 4.3.3.1. Use the **Densitometry > Densitometry plot** to calculate the density profile. Create
470 and plot a density profile by selecting **Overlay Plotting > Plot Density Profile**, that can serve as
471 an overlay on the core planes.

472
473 4.3.3.2. For MXD calculation, indicate a percentage (recommended is 20%, e.g., 20%
474 means that only 20% of the highest density values are included), to exclude lower values from
475 resin ducts, earlywood etc. For mean density values use 100%. For minimum density also use
476 100%.

477
478 4.3.4. Perform manual and/or automatic indication of ring boundaries using one left mouse click
479 per ring as described below.

a mis en forme : Police :Gras

480
481 NOTE: Insert the same green bars as in step 4.3.2.1., this time the user should do this now ring
482 per ring (Figure 9).

483
484 4.3.4.1. Use **Overlay Plotting > Plot Density Profile**, to visualize the density profile on the
485 image. It is best to indicate the ring boundary also in conjunction with this profile.

486
487 ~~4.3.4.2. For a first visual crossdating as a quality tool with other cores from the same tree,~~
488 ~~open another RingIndicator application (by double-clicking on the icon again) and visually assess~~
489 ~~both cores on the screen (use two monitors for this approach, if needed).~~

a mis en forme : Non Surlignage

Commenté [A1]: @ Jove, we removed this because
redundant an confusing

490
491 NOTE: Open as many instances from the toolboxes as needed, in combination with the
492 CoreComparison toolbox (see further).

493
494 ~~4.3.4.3.4.3.4.2.~~ For automatic indications (~~recommended for conifers for instance works~~
495 ~~best for boreal conifers or species with clear ring boundaries defined by the density profile~~), use
496 **Auto > Max/Min/Inflection** detection. This function will automatically indicate tree ring
497 boundaries based on the densitometry profile created in the previous step.

498
499 ~~4.3.4.4.4.3.4.3.~~ Depending on the species (diffuse-porous/ring-porous or coniferous), use
500 the maximum/minimum, or curve inflection (right after the density peak) as the tree ring
501 boundary criterion. The first parameter is a threshold for the peak function, the second is a
502 smoother function.

503
504 ~~4.3.4.5.4.3.4.4.~~ After performing the automated ring indications, perform automatic
505 shifting (with window size of 1 pixel and user-defined iterations), while making sure that no ring
506 indications are plotted on each other due to this shift and re-run the densitometry plot.

507
508 ~~4.3.4.6.4.3.4.5.~~ Change the felling date to the year at which the increment cores were
509 taken on living trees or any other date that is suitable. The default is set at 1900.

510
511 ~~4.3.4.7.4.3.4.6.~~ In **Overlay Plotting**, select **Plot Rings** and the years are displayed. Also
512 select the plane on which to plot rings and/or the density profile.

513
514 ~~4.3.4.8.4.3.4.7.~~ ~~After pairwise~~ Once there are two cores fully indicated (e.g. from the same
515 ~~tree), do pairwise~~ comparisons of core ring width curves by opening the ~~s~~ are done in the
516 CoreComparison toolbox. ~~Open one~~ import the rings in RingIndicator ~~instance per core and~~
517 ~~change the positions and the angles of the green bars, and export them again~~ edit the indications
518 ~~if necessary. Export them again and~~ Carry out this iterative and interactive approach to obtain
519 good quality data. In the next step (step 4.4.1.), do this for all cores ~~and to~~ export both the TRW
520 data and the corresponding density values.

521

522 [4.3.4.9-4.3.4.8.](#) Check double-ring positions in the **Rings > Check Rings** option; this will
523 check if any indications have a position outside the images as well as rings that are overlapping,
524 e.g., in case if a double click has happened twice on the same position.

525
526 [Place **Figure 9** here]

527
528 [4.3.4.10-4.3.4.9.](#) For broken cores that have bits and pieces, start at the pith, and demarcate
529 anomalies (air due to broken cores, bright spots due to resin pockets, contamination, and
530 decayed parts) as if they were rings to delete them afterwards. End with the tree ring boundary
531 of the last formed ring and export the data. Try to avoid broken cores during sampling (see step
532 1.1. and³⁹).

533 NOTE: Both missing as well as broken rings can be indicated, see **Figure 10**. Two cases are defined,
534 one where the core breaks in the middle of a ring, and one where the core breaks on a ring
535 boundary. For the first one (**Figure 10A**), indicate the gap, note the number of that gap followed
536 by number 1 and close the brackets. Then the program will sum the two parts of the ring to obtain
537 a correct TRW value. In the second case, the gap number is indicated followed by number 2, and
538 the program will omit this section (**Figure 10B**).

539
540 [Place **Figure 10** here]

541
542 [4.3.4.11-4.3.4.10.](#) By indicating rings and generating density profiles, several .txt files are
543 created and stored in the same folder as the multipage TIFF files, and the most important ones
544 are listed in **Table 1**. Keep these .txt files in the same folder as the TIFF images, in order to further
545 treat them in the CoreComparison module (step 5.4).

546
547 [Place **Table 1** here]

548
549 NOTE: For calculating density values in a different way based on the raw density profile (for
550 instance latewood width vs earlywood width⁴⁵ or other metrics not supported by the
551 CoreComparison and CoreProcessor toolboxes), use the zpos_corr and density_corr text files and
552 use these for such calculations.

553
554 **4.4. For comparing TRW series and exporting the data via CoreComparison, follow the steps**
555 **below.**

556
557 NOTE: When using this toolbox, two windows are opened simultaneously, the CoreComparison
558 and the PatternMatching module. The CoreComparison deals with cross dating and exporting of
559 TRW data, whereas the PatternMatching module can be used for exporting density profiles and
560 density values (MXD, minimum density (MND), mean density, and 4 quartile values). The main
561 goal of CoreComparison is to generate the TRW values and density values, and to have a rough
562 estimate of cross dating quality, with a coupling to RindIndicator in case of missing rings or wrong
563 indications.

564

565 4.4.1. Perform the following steps for cross dating or matching the ring width pattern with other
566 series.

567
568 4.4.1.1. Once the RingIndicator section is completed, select the text files to compare, and
569 a screen will open with ring widths, together with cross dating or ~~data~~-statistical parameters such
570 as Gleichläufigkeit (GLK)⁴⁶ and or Spearman correlation between individual series (**Figure 11**).

571
572 4.4.1.2. To make changes in the ring indications (for instance during cross dating), re-open
573 **RingIndicator**, import data, export the modified data and rings, and use the **Refresh** button in
574 the CoreComparison module (see top left-hand side) to see the changes.

575
576 4.4.1.3. Open multiple RingIndicator instances to make changes to different tree-ring
577 series and use multiple screen monitors to do this.

578
579 4.4.1.4. In the GLK and CORR menu (**Figure 11**), perform a series of actions ~~scan~~-related to
580 GLK, such as thresholding the figures, exporting the GLK values to a spreadsheet and ranking
581 them.

582
583 [Place **Figure 11** here]

584
585 4.4.2. Obtain TRW values from CoreComparison as described below.

586
587 4.4.2.1. Use the plotting and exporting function in CoreComparison to change the offset
588 between the curves (a larger value result in a smaller offset). Visualize the TRW data in a plot by
589 selecting **Plotting and Exporting > Ring width**.

590
591 4.4.2.2. Export the TRW data by clicking **Plotting and Exporting > Export RW Data**. Export
592 ring widths in spreadsheet format or in Tucson format.

593
594 4.4.3. Obtain the density profiles from the PatternMatching module as described below.

595
596 4.4.3.1. To obtain the density profiles in spreadsheet, go to the **PatternMatching** module,
597 and after loading in the profiles, in the menu **Other** select **Plotting > Export Corrected Density**
598 **Profiles**.

599
600 4.4.3.2. To batch process profiles or to assess the data using different methods, directly
601 use the .txt files from **Table 1** (see step 4.3.4.11).

602
603 4.4.4. Obtain MXD, MND, and other density values from the PatternMatching module as
604 described below.

605
606 4.4.4.1. For obtaining the mean density, MXD, MND and quartile data per tree ring, use
607 **Other Plotting > Export Clustered Data**. A spreadsheet will be generated and can be found in the
608 same folder as the TIFF files.

609
610 4.4.4.2. The tabs in the spreadsheet are detailed in **Table 2**. Export these tree ring variables
611 to Rstudio or other software for further analysis.

612
613 [Place **Table 2** here]

614 **REPRESENTATIVE RESULTS:**

615 If the goal is biomass estimation or tree growth increment of many samples, i.e., inter-ring scale
616 (**Figure 1**), then sample holder 1 (**Figure 3B**) is used to scan samples to obtain density profiles
617 (see step 5.4.3) and estimates of tree growth, e.g., for fast-growing trees with large TRW, which
618 allows for a coarser resolution. **Figure 12** shows an example of both pith-to-bark and axial density
619 trends of *T. superba*, a species from the Congo basin. These diagrams were based on scans of 110
620 μm from tree increment cores taken at various height positions in the stem. ~~The profiles were~~
621 ~~replicated to 360° to mimic the stem cross-section.~~

622
623 For the ring scale (**Figure 1**) that is targeted by scanning cores with sample holder 2 (**Figure 3B**),
624 tree ring analysis and MXD calculation is possible. An example of this is **Figure 13**, where a
625 minimum density (in this case linked to wet season precipitation) and maximum density
626 chronology is plotted for cores of *W. cedarbergensis* from the Cedarberg mountains in South
627 Africa²⁶. This was based on scanning mounted cores at 10 μm resolution.

628
629 For the anatomical scale (**Figure 1**), scanning cores with holder 3, i.e., multiple cores in papers
630 straws wrapped together, allows a resolution of up to 4 μm . The result is shown in **Figure 14**,
631 ~~where~~ ~~are~~ ~~of~~ ~~an~~ ~~X-ray~~ ~~CT~~ ~~image~~ ~~slice~~ ~~of~~ ~~oak~~ ~~core~~ (*Q. robur*) ~~scanned~~ ~~is~~ ~~shown~~, ~~and~~ ~~with~~ both
632 earlywood and latewood vessels ~~are~~ segmented.

633 **FIGURE AND TABLE LEGENDS:**

634
635 **Figure 1: General methodological decision tree for X-ray CT scanning.** The rows indicate the
636 steps to take, starting from the research goal all the way to the final data format. White boxes
637 are the steps that are relevant for this toolchain. Greyed-out boxes are steps that can be
638 performed with other software or R packages, such as dplr⁴⁷ and Treeclim⁴⁸ for tree-ring analysis,
639 and ROXAS⁴⁴ as well as ImageJ⁴² or other (commercial) applications for deriving wood anatomical
640 parameters based on the CT images.

641
642 **Figure 2. The HECTOR scanning system.** The system³⁵, showing the source detector distance
643 (SDD) and the source object distance (SOD).

644
645 **Figure 3. Scanning set-up examples.** (A) A log, (B) a cello⁴⁹, (C) sample holders (type 1) with tree
646 cores for batch scanning and (D) sample holder type 2 with increment cores for helical scanning
647 mounted on the rotation stage of HECTOR.

648
649 **Figure 4. Workflow for extracting and drying cores prior to scanning.** (A) Increment cores in
650 paper straws are first put in a hot water bath and subsequently in a hot ethanol-toluene mixture
651 with a Soxhlet apparatus for 24 h then dried, put in a hot water bath for 24 h, then dried again at
652

653 103.5 °C, then conditioned before scanning. (B) Images of the adapted Soxhlet set-up at Ghent
654 University. Multiple adapted Soxhlet apparatuses are serially attached. Note the aluminum foil
655 and insulation tubes to keep the solvent or solvent mixture warm enough for extraction. (C)
656 Example of the surface of an increment core from *Pinus longaeva* before and after extraction.
657 Resins and other extractives mask the true density signal, due to attenuation of the X-rays.

658
659 **Figure 5. Sample holder types and resolution.** Design of the three main holder types, that
660 correspond to (A) inter-ring scale, (B) tree ring scale, and (C) anatomical scale. Due to the
661 resulting size, the number of samples decreases with increasing resolution requirement. The
662 corresponding 3D renderings for (D) inter-ring scale (holder 1), (E) ring width and MXD (holder
663 type 2), and (F) anatomical parameters (holder type 3). Scale bar = 5 mm.

664
665 **Figure 6. Obtaining wood density values from grey values.** Conversion of the grey values of the
666 image to actual wood density estimates, through calibration with a reference material. (A)
667 Sample holder 1, at 60 µm resolution, showing the air reference, white reference, and the core.
668 (B) Sample holder, at 20 µm, air, reference and core are shown as well.

669
670 **Figure 7. Pre-processing steps of the 3D core volume.** Pre-processing steps of the 3D core
671 volume before visualization and indicating the tree rings. (A) Tilt (axial) correction, (B) tangential
672 correction, with the grey bars indicating the fiber direction, (C) the cropping of the core volume
673 to obtain a volume with only woody voxels.

674
675 **Figure 8. Screenshot of a core.** Screenshot of a core (tangential width approximately 3 mm,
676 resolution µm) opened in the RingIndicator toolbox. Top pane is the transversal view, bottom
677 pane is the radial view. Green bars show the structure indication (in this case on the ring
678 boundary), red line is the density profile. The yellow numbers indicate calendar year, and the
679 blue numbers the generic ring number (counting from the first indication).

680
681 **Figure 9. Ring indication and density profile visualization in the RingIndicator module.** Green
682 lines are indications, red line is the wood density profile.

683
684 **Figure 10. How to deal with cracks in the cores.** (A) When a crack occurs in the middle of the
685 ring, the generic ring number (starting from the pith) will be labeled as a gap, and TRW will be
686 calculated by summing the widths of the two ring parts. Parameters are 3 (number of the ring)
687 and 1 (case 1: crack in the middle of a ring)³¹. (B) When a crack occurs at a ring boundary, the gap
688 will be omitted from the TRW calculation. Parameters are 3 (number of the ring) and 2 (case 2:
689 crack at the ring boundary)³¹.

690
691 **Figure 11. Screenshot of the Core-Comparison module.** Screenshot of the Core-Comparison
692 module, where ring width series are distributed vertically for easy visual comparison. The top
693 pane shows the ring width of 4 cores from *Fagus sylvatica*, the bottom left pane shows the
694 Gleichläufigkeit (GLK) and the bottom right pane the Spearman correlation coefficient.

695
696 [Place **Figure 12** here]

697
698 **Figure 12. Radial and axial wood density trends.** (A) Radial variation of oven-dry wood density
699 (kg/m^3) from 46 *Terminalia superba* trees from the Congo Basin (DRC; data extracted from ²¹),
700 with minimum (min), mean, and maximum (max) values, with a raw profile (red) superposed. (B)
701 Radial variation and axial variation from a single *Terminalia superba* tree, where at every height
702 interval of 1 m an increment core was taken and scanned (23 cores in total). There is an increasing
703 density trend from pith to bark (highlighted according to colorbar), and a higher wood density in
704 the upper stem. Scanning resolution = 110 μm .

705
706 [Place **Figure 13** here]

707
708 **Figure 13. Chronology development.** A minimum density and a maximum latewood density
709 chronology from *Widdringtonia cedarbergensis*. Data extracted from²⁶. The scanning resolution
710 is 10 μm .

711
712 [Place **Figure 14** here]

713
714 **Figure 14. High-resolution scan of an oak sample.** Example of a segmentation of oak vessels on
715 *Quercus robur* scanned at 4 μm . Segmentation was performed using the **octopus-Octopus** analysis
716 software, using bilateral filtering, thresholding, a series of binary shrink and expand operations,
717 Euclidian distance transform and finally labelling of the vessels. Color represents vessel size,
718 ranging from small (dark blue) to large (dark orange).

719
720 **Table 1. Text files generated by RingIndicator.** List of text files that are generated after indicating
721 rings and exporting the density profile in RingIndicator. These should remain in the same folder
722 as the .tiff file in order to be opened in the CoreComparison module.

723
724 **Table 2. Spreadsheet details.** Tree-ring variables that are exported into a spreadsheet named
725 export clustered data.

726 727 **DISCUSSION:**

728 Critical steps within the protocol

729 Critical steps within the protocol include proper handling of the increment borer to obtain high-
730 quality increment cores (step 1.1. and see³⁹) to avoid bits and pieces. Next, it is essential that
731 cores be left unmounted (but see²⁶), both for insertion in the sample holder (**Figure 5**, see²¹) as
732 well as for proper resin extraction⁵⁰ and for possible future analysis. Right before scanning (step
733 2.2.2.), the conditioning of the samples in the scanner room is essential to avoid dimensional
734 deformations due to fluctuations of moisture. Then, once the rings can be indicated in the
735 RingIndicator module, it is essential that the ring boundaries and angles are well indicated,
736 because the density peaks can flatten, similar to inaccurate indications on classic densitometry
737 systems¹³. The indication is also important to have correct ring width measurements²¹. The next
738 critical step is proper cross dating through the CoreComparison module (step 4.4.1.). If some
739 cores have wrong indications, there could be an environmental signal loss⁵¹.

740

741 Modifications and troubleshooting of the technique

742 Note that the toolboxes RingIndicator and CoreComparison can be used for flatbed images as
743 well⁵² after converting the data to the proper resolution in the CoreProcessor module. Therefore,
744 the DICOM format (common format for medical purposes and is also used for wood samples⁵³)
745 needs to be converted to .tiff. Departments that have medical CT scanner can use this software
746 only for the type 1 inter-ring scale analysis type with resolutions of around 200 µm.

747

748 Limitations of the technique

749 The drawback of the method is that there are only a few facilities available for scanning for tree
750 ring scale and anatomical scale (which require a higher resolution). Data volume and data
751 handling can be challenging for larger volumes⁵⁴. For very high-resolution images of wood
752 anatomy (e.g., to further elucidate pit structures etc.), refer to classic procedures of using
753 microsections for classic transmitted light microscopy²⁷, scanning electron microscopy or
754 confocal microscopy⁵⁵.

755

756 Significance with respect to existing methods

757 Using the X-ray CT₇ toolchain for increment cores presented here has been tested as showing
758 reliable density values due to the calibration step (**Figure 6**)⁵⁶ and generates MXD values
759 comparable to existing densitometry procedures¹³.

760

761 Future applications of the technique

762 Any future applications of the technique might include the application of deep learning, amongst
763 others [for](#) the automated ring indications and wood tissue quantifications⁵⁷, specifically for 3D
764 images.

765

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774

775 DISCLOSURES

776 The authors have no conflicts of interest to disclose.

777

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a mis en forme : Néerlandais (Belgique)

a mis en forme : Français (France)

