

1 **TITLE:**
2 Tree Core Analysis with X-ray Computed Tomography

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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 μ CT scans. When the ultrastructure of the cell walls needs to be visualized, scanning electron
91 microscopy is the most commonly used method²⁷. At the anatomical scale, the individual tissue
92 fractions become visible so that physiological parameters can be derived from the images. Based
93 on the individual anatomical parameters and the cell wall density of wood, anatomical density
94 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. 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]

126 127 **X-CT research on wood**

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]

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

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

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

149
150 Practically, the object is mounted on the rotation stage, a proper SOD and SDD are selected, a
151 certain power is selected as well (related to object size, density, and composition) and the object
152 is rotated 360° and during that rotation multiple projections are taken. These projections are
153 then used to reconstruct the interior structure of the object. There are several reconstruction
154 algorithms available, of which the most used ones are still based on the analytical framework
155 developed decades ago, relying on the Radon transformation and the Fourier slice theorem. For
156 more details, the reader is referred to specialized literature³⁶.

157
158 **Conundrum of resolution, data volume and sample size:** Resolution is key in X-ray CT scanning.
159 In systems with inverse geometry, or parallel beam geometry such as synchrotron beamlines,
160 other considerations play a role. This protocol only discusses standard lab-based X-ray CT
161 scanning with cone-beam geometry. Here, the concept of magnification, detector pixel size and
162 spot size are essential. Magnification is defined as the ratio of SDD/SOD. Next, the pixel size of
163 the detector obviously also impacts the resolution: the smaller the pixel size, the higher the
164 resolution, but in most cases the field of view (FoV) is also directly related to pixel size and size
165 of the detector (smaller pixel size, smaller FoV for the same number of pixels). Further, the spot
166 size of the X-ray beam is also important: the larger the spot size, the lower the resolution, which
167 means that less details can be seen.
168

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

175 In most cases, however, the voxel size at which an object can be scanned is mostly limited by the
176 size of the object. This means that the larger the object, the lower the voxel size will be. If the

177 object does not fit within the FoV of the detector for a certain voxel size, then the voxel size can
178 be reduced, e.g., by limiting the magnification.

179
180 Scan time and data volume are important to consider when deciding the desired voxel size. In
181 general, the smaller the voxel size means the higher the detail one wants to see, the smaller the
182 sample or the fewer samples that can be scanned at once, more time is required and larger the
183 data volumes will be collected. Imagine the following theoretical example: one can scan a sample
184 measuring 10 cm x 10 cm x 10 cm at 50 μm at once with a certain X-ray CT system and would like
185 to scan that same volume at 10 μm , the volume that fits within the FoV would only be 2 cm x 2
186 cm x 2 cm, assuming that this is physically possible. This means that 125 scans are needed ($5^3 =$
187 5 times higher resolution, scales to the power of 3 due to the volumetric nature of the imaging
188 technique) to cover the entire volume, and that the data volume would increase likewise. Of
189 course, this is only a thought experiment, and one needs to consider much more than only
190 resolution. For more information, the reader is referred to an overview of the scanning
191 possibilities³⁷.

192
193 **Flexibility of instruments for scanning of wooden objects:** In the last decade, many companies
194 have delivered X-ray CT systems with a similar assembly as HECTOR³⁵. An overview of several CT
195 systems, especially assessed for their temporal resolution, is given in³⁸.

196
197 In all, the flexibility and user-friendliness of X-ray CT systems has improved considerably. Many
198 systems allow scanning of a diverse range of objects, which is also the case with the systems at
199 UGCT. The protocol below is demonstrated for the HECTOR system, which is suitable for tree-
200 ring analysis. The protocol however is valid for any other available system if resolution and data
201 format allows for it.

202
203 These systems allow the scanning of a variety of objects. A few pictures of different wooden
204 objects scanned with the HECTOR system are given in **Figure 3**. It is this flexibility that comprises
205 the three scales we present in **Figure 1**, ranging from a coarse resolution to a very fine resolution.

206
207 [Place **Figure 3** here]

208
209 **PROTOCOL:**

210
211 **1. Core sampling**

212
213 **1.1. Sample the tree with a Pressler borer.** Consult references on how to core a tree
214 manually³⁹. This toolchain is presented for 5.15 mm cores.

215
216 **1.2. Put the tree cores unglued into 6 mm paper straws or in any other receptor that does not**
217 **require glue.** Do not glue the tree cores on a wooden support. If the cores were already mounted,
218 unmount them with scalpel/saw or solvent depending on the glue type.

219

220 1.3. Avoid wrapping them in plastic tubes, try to dry them first to avoid mold growth and
221 fungal decay.

222 1.4. Use pencil to write on the paper straws, since the extraction could fade marker writings.
223

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

228 2. Core preparation before scanning 229

230 2.1. Perform an ethanol/toluene or ethanol or acetone⁴⁰ Soxhlet extraction for at least 24 h
231 (Figure 4A,B). This is necessary to ensure a resin-free wood volume to have an accurate density
232 calculation (resin attenuates the signal, Figure 4C^{13, 41}).
233

234 [Place Figure 4 here]
235

236 2.1.1. Make a solution of 1000 mL of ethanol with 427 mL of toluene (according to e.g., ASTM
237 D 1107 – 96) or pure ethanol or acetone. Many universities have a lab glass workshop that can
238 make an adapted version of the Soxhlet apparatus for tree cores (Figure 4B).
239

240 2.1.2. Fill the round-bottomed flask with the solvent, and put the samples within the straws in
241 a stainless-steel support in the Soxhlet apparatus with cooler on a laboratory heater. Allow for at
242 least 6 siphonings per h.
243

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

251 2.1.3. Check the temperature and number of siphonings regularly. Dry the cores under a
252 chemical hood (check lab policy for this) to remove the solvent residue or put a drying oven under
253 the chemical hood.
254

255 2.2. Dry the cores for 24 h in a drying oven (103.5 °C) under exhaust ventilation and perform
256 a hot water bath (90 °C) or hot water Soxhlet extraction for 24 h as described above.
257

258 2.2.1. Keep the samples in the paper straws in the steel sample holders. Dry again in a dry oven
259 for 24 h at 103.5 °C, then condition to 60% relative humidity (RH) before scanning.
260

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

- 264
- 265 2.2.2. In case the water extraction degenerates the paper straws, put the tree cores back into
- 266 new 6 mm paper straws²¹. Both 5.15 mm and 4 mm cores can be put into 6 mm paper straws.
- 267
- 268 2.2.3. Make sure the entire paper straw is filled with wood. Wrap both straw-ends up and cut
- 269 the ends. This enables easy insertion of the straws in the cylindrical holder.
- 270
- 271 2.2.4. Make sure the cambium (bark) side is clearly indicated on the core because the
- 272 CoreProcessor and RingIndicator assume that the bark side is down. Remove bits and pieces as
- 273 well as cores with cracks as these are difficult to work with.
- 274

275 **3. Core scanning**

276

277 3.1 Select the proper sample holder type (**Figure 5**) as described below, depending on the

278 research purpose as described in **Figure 1**.

279

280 3.1.1 For inter-ring scale purposes, use holder type1 – cardboard and perforated floral foam.

281

282 3.1.2 For the MXD data use holder type 2 – a plastic sample holder that holds 6 cores of 1–15

283 cm length and has a 3 cm diameter.

284

285 3.1.3 Use holder type 3 – for dendro-anatomy purposes that consists of simply tying paper

286 straws together.

287

288 [Place **Figure 5** here]

289

290 3.2 Load the tree cores in paper straws into the sample holder and fill in the spreadsheet

291 template that can be downloaded on <https://dendrochronomics.ugent.be/#software>. Each

292 number corresponds to a given position in the sample holder.

293

294 3.3 Scanning in an X-ray μ CT facility: consult an X-ray CT expert for the proper settings and

295 scanning protocol. For the scanner used in this protocol, see ³⁷.

296

297 3.4 Reconstruction of the scanned images: consult an X-ray CT expert for the proper settings

298 of the reconstruction, or the settings that come with the CT scanner (software package of the X-

299 ray CT scanner). For reconstruction parameters for this protocol, see ³⁷.

300

301 **4. Obtain TRW data, density data and anatomical data**

302

303 4.1. Getting started with the software.

304

305 4.1.1. To obtain density values (holder type 1 and 2), install the following three software

306 packages: (i) CoreProcessor, (ii) RingIndicator and (iii) CoreComparison toolboxes from

307 www.dendrochronomics.ugent.be.

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

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

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

4.2 Preprocessing the core volumes with the CoreProcessor.

NOTE: For high-resolution anatomy data, following processing steps are not necessary (**Figure 1**), once the reconstructed volumes are available, refer to the ImageJ guidelines for 3D images⁴². Segmentation can be done through the Weka plugin⁴³ for indicating vessels on individual slices for instance⁴², or in software plugins such as ROXAS⁴⁴.

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

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

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

4.2.1.3 Select the 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, check a few horizontal slices to ensure if all cores and references were correctly mounted into the cylinder.

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

4.2.1.5 For sample holder 1, inspect the multiscreen with 9 cross-sections, generated through the reference material to check whether the density calculation was performed on the wood (and not on the paper straw or other non-wood material). This is essential for correct conversion to actual wood density values.

[Place **Figure 6** here]

351 4.2.1.6 In a new pop-up window, select every core separately by drawing a circle/ellipse around
352 it. Perform labelling based on the information from the spreadsheet file, check whether the name
353 is correct. Respect the order in which the template is filled. All info is stored in a MAT file, that
354 will be used during the next step for extracting all cores at once.

355
356 4.2.2 Click on **Mass Core Extraction** to carry out core extraction for all the cores in a given
357 cylinder (that were indicated in 4.2.1.2), given that the metadata and spreadsheet files are
358 located in the same folder.

359
360 NOTE: When using Mass core extraction, all cores will be extracted according to the labelling of
361 the user (that are stored in the mat file folder). As a result of this operation, a new folder named
362 **Extracted** will be created which contains all the separate cores. When multiple sample holders
363 are prepared in the first step, and all the MAT files are stored in the same folder, all MAT files in
364 this folder will be processed.

365
366 4.2.3. In the CoreProcessor toolbox, click on **Manual Tg Correction**, then select the **Extracted**
367 folder to ensure proper orientation of the transversal and radial plane of every core volume,
368 similar to mounting physical samples in a sample holder before sanding keeping the transversal
369 plane in view (**Figure 7B**).

370
371 4.2.3.1. Some cross-sections are displayed so that the user can see the grain direction. In
372 the last plot (bottom right), inspect the slice that is presented and draw a line to indicate the
373 grain direction. Double click to automatically rotate the core.

374
375 4.2.3.2. Then a screen appears, here crop the volume of the core (**Figure 7C**), making sure
376 that the rectangle encompasses the entire core diameter. This is necessary because the central
377 point of the rectangle should be as close as possible to the core axis (for correct alignment in the
378 RingIndicator module).

379
380 [Place **Figure 7** here]

381
382 NOTE: If scanning at high resolution, select only material within the core at this stage and use the
383 converter to switch to sample holder type 2. This is certainly helpful for ring-porous species for
384 example.

385
386 4.2.4. Perform the following optional steps in case of misaligned, flipped, or broken cores or
387 incompatible image formats.

388
389 4.2.4.1. Core tilt correction (only for sample holder 1): To automatically correct for core
390 tilt (e.g., when the core is slightly inclined in the cylinder; **Figure 7A**). This is important for later
391 steps. Select the **Extracted** folder created in the previous step.

392
393 NOTE: This is normally not needed if you have a sample holder type 2 or type 3 (**Figure 5**) or when
394 all samples and sample holders are put perfectly upright.

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

4.2.4.3. Glue cores: Stitch large parts of individual cores together virtually. The idea is not to stitch perfectly, but to make sure that, if needed, single cores are in the same multipage TIFF volume. Use the broken core indication (see step 4.3.4.9) to overcome the spaces in between the core pieces.

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

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

4.3. Correcting the angles and indicating rings in RingIndicator.

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

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

4.3.1.1. To get familiar with the GUI, open the toolbox and select a multipage **TIFF File** (one that was created after extraction and after tg corrected, so from the folder name **Tg corrected**).

4.3.1.2. Select the **Half Thickness of Slice Image** to display an average image of the central X slices, with X ranging from 1-10 with 1 being no averaging (i.e. only display the central slice), and 10 being an average image of the 10 central slices.

NOTE: Averaging can be beneficial to highlight ring borders better, and suppress noise, on the other hand it can also make ring borders look less distinct, especially when the fiber angle is substantial. Please tune this value according to the needs. It is important to mention that this is merely for displaying, it does not impact the underlying volume, nor does it impact the eventual calculations/results.

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

439 [Place **Figure 8** here]

440

441 4.3.1.4. To load a new core volume, search for the **Volume > Load Volume** function to
442 select a new multipage **TIFF file** in the directory. Now the user is ready to indicate the structure
443 direction to subsequently indicate the actual tree rings.

444

445 4.3.2. **Structure correction to facilitate automatic tree ring indications.**

446

447 4.3.2.1. Manually click on the image to insert green bars for structure correction. Start
448 with the pith (or oldest ring available) and end with the most recent ring. Along the core correct
449 for structural deviations on both the transversal and the radial plane.

450

451 4.3.2.2. Make sure to indicate the ring and fiber angle properly, because all subsequent
452 indications will take an interpolated angle between the respective indications.

453

454 4.3.2.3. Insert a green bar on one plane, it will automatically generate one on the other
455 plane. After inserting the bar, move the nodes (white squares) at the end of the bar to change
456 the angle. Use the middle node to change the bar position. Adjust the size of the nodes for ease
457 of use.

458

459 4.3.2.4. After every step, make sure to press **Data > Export** and **Rings > Export Rings**, to
460 ensure that ring and fiber indications are written to the proper .txt files. Throughout all
461 corrections, make sure to always perform these two actions.

462

463 4.3.3. **Calculating the density profile.**

464

465 4.3.3.1. Use the **Densitometry > Densitometry plot** to calculate the density profile. Create
466 and plot a density profile by selecting **Overlay Plotting > Plot Density Profile**, that can serve as
467 an overlay on the core planes.

468

469 4.3.3.2. For MXD calculation, indicate a percentage (recommended is 20%, e.g., 20%
470 means that only 20% of the highest density values are included), to exclude lower values from
471 resin ducts, earlywood etc. For mean density values use 100%. For minimum density also use
472 100%.

473

474 4.3.4. Perform manual and/or automatic indication of ring boundaries using one left mouse click
475 per ring as described below.

476

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

479

480 4.3.4.1. Use **Overlay Plotting > Plot Density Profile**, to visualize the density profile on the
481 image. It is best to indicate the ring boundary also in conjunction with this profile.

482

483

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

486

487 4.3.4.2. For automatic indications (works best for boreal conifers or species with clear ring
488 boundaries defined by the density profile), use **Auto > Max/Min/Inflection** detection. This
489 function will automatically indicate tree ring boundaries based on the densitometry profile
490 created in the previous step.

491

492 4.3.4.3. Depending on the species (diffuse-porous/ring-porous or coniferous), use the
493 maximum/minimum, or curve inflection (right after the density peak) as the tree ring boundary
494 criterion. The first parameter is a threshold for the peak function, the second is a smoother
495 function.

496

497 4.3.4.4. After performing the automated ring indications, perform automatic shifting (with
498 window size of 1 pixel and user-defined iterations), while making sure that no ring indications are
499 plotted on each other due to this shift and re-run the densitometry plot.

500

501 4.3.4.5. Change the felling date to the year at which the increment cores were taken on
502 living trees or any other date that is suitable. The default is set at 1900.

503

504 4.3.4.6. In **Overlay Plotting**, select **Plot Rings** and the years are displayed. Also select the
505 plane on which to plot rings and/or the density profile.

506

507 4.3.4.7. Once there are two cores fully indicated (e.g. from the same tree), do pairwise
508 comparisons of core ring width curves by opening the CoreComparison toolbox. Open one
509 RingIndicator instance per core and edit the indications if necessary. Export them again and array
510 out this iterative and interactive approach to obtain good quality data. In the next step (step
511 4.4.1.), do this for all cores to export both the TRW data and the corresponding density values.

512

513 4.3.4.8. Check double-ring positions in the **Rings > Check Rings** option; this will check if
514 any indications have a position outside the images as well as rings that are overlapping, e.g., in
515 case if a double click has happened twice on the same position.

516

517 [Place **Figure 9** here]

518

519 4.3.4.9. For broken cores that have bits and pieces, start at the pith, and demarcate
520 anomalies (air due to broken cores, bright spots due to resin pockets, contamination, and
521 decayed parts) as if they were rings to delete them afterwards. End with the tree ring boundary
522 of the last formed ring and export the data. Try to avoid broken cores during sampling (see step
523 1.1. and³⁹).

524 NOTE: Both missing as well as broken rings can be indicated, see **Figure 10**. Two cases are defined,
525 one where the core breaks in the middle of a ring, and one where the core breaks on a ring
526 boundary. For the first one (**Figure 10A**), indicate the gap, note the number of that gap followed

527 by number 1 and close the brackets. Then the program will sum the two parts of the ring to obtain
528 a correct TRW value. In the second case, the gap number is indicated followed by number 2, and
529 the program will omit this section (**Figure 10B**).

530

531 [Place **Figure 10** here]

532

533 4.3.4.10. By indicating rings and generating density profiles, several .txt files are created
534 and stored in the same folder as the multipage TIFF files, and the most important ones are listed
535 in **Table 1**. Keep these .txt files in the same folder as the TIFF images, in order to further treat
536 them in the CoreComparison module (step 5.4).

537

538 [Place **Table 1** here]

539

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

544

545 4.4. For comparing TRW series and exporting the data via CoreComparison, follow the steps
546 below.

547

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

555

556 4.4.1. Perform the following steps for cross dating or matching the ring width pattern with other
557 series.

558

559 4.4.1.1. Once the RingIndicator section is completed, select the text files to compare, and
560 a screen will open with ring widths, together with cross dating or statistical parameters such as
561 Gleichläufigkeit (GLK)⁴⁶ and or Spearman correlation between individual series (**Figure 11**).

562

563 4.4.1.2. To make changes in the ring indications (for instance during cross dating), re-open
564 **RingIndicator**, import data, export the modified data and rings, and use the **Refresh** button in
565 the CoreComparison module (see top left-hand side) to see the changes.

566

567 4.4.1.3. Open multiple RingIndicator instances to make changes to different tree-ring
568 series and use multiple screen monitors to do this.

569

570 4.4.1.4. In the GLK and CORR menu (**Figure 11**), perform a series of actions related to GLK,
571 such as thresholding the figures, exporting the GLK values to a spreadsheet and ranking them.

572
573 [Place **Figure 11** here]

574
575 4.4.2. Obtain TRW values from CoreComparison as described below.

576
577 4.4.2.1. Use the plotting and exporting function in CoreComparison to change the offset
578 between the curves (a larger value result in a smaller offset). Visualize the TRW data in a plot by
579 selecting **Plotting and Exporting > Ring width**.

580
581 4.4.2.2. Export the TRW data by clicking **Plotting and Exporting > Export RW Data**. Export
582 ring widths in spreadsheet format or in Tucson format.

583
584 4.4.3. Obtain the density profiles from the PatternMatching module as described below.

585
586 4.4.3.1. To obtain the density profiles in spreadsheet, go to the **PatternMatching** module,
587 and after loading in the profiles, in the menu **Other** select **Plotting > Export Corrected Density**
588 **Profiles**.

589
590 4.4.3.2. To batch process profiles or to assess the data using different methods, directly
591 use the .txt files from **Table 1** (see step 4.3.4.11).

592
593 4.4.4. Obtain MXD, MND, and other density values from the PatternMatching module as
594 described below.

595
596 4.4.4.1. For obtaining the mean density, MXD, MND and quartile data per tree ring, use
597 **Other Plotting > Export Clustered Data**. A spreadsheet will be generated and can be found in the
598 same folder as the TIFF files.

599
600 4.4.4.2. The tabs in the spreadsheet are detailed in **Table 2**. Export these tree ring variables
601 to Rstudio or other software for further analysis.

602
603 [Place **Table 2** here]

604
605 **REPRESENTATIVE RESULTS:**

606 If the goal is biomass estimation or tree growth increment of many samples, i.e., inter-ring scale
607 (**Figure 1**), then sample holder 1 (**Figure 5**) is used to scan samples to obtain density profiles (see
608 step 5.4.3) and estimates of tree growth, e.g., for fast-growing trees with large TRW, which allows
609 for a coarser resolution. **Figure 12** shows an example of both pith-to-bark and axial density trends
610 of *T. superba*, a species from the Congo basin. These diagrams were based on scans of 110 μm
611 from tree increment cores taken at various height positions in the stem.

612

613 For the ring scale (**Figure 1**) that is targeted by scanning cores with sample holder 2 (**Figure 5B**),
614 tree ring analysis and MXD calculation is possible. An example of this is **Figure 13**, where a
615 minimum density (in this case linked to wet season precipitation) and maximum density
616 chronology is plotted for cores of *W. cedarbergensis* from the Cedarberg mountains in South
617 Africa²⁶. This was based on scanning mounted cores at 10 µm resolution.

618
619 For the anatomical scale (**Figure 1**), scanning cores with holder 3, i.e., multiple cores in papers
620 straws wrapped together, allows a resolution of up to 4 µm. The result is shown in **Figure 14**,
621 where an X-ray CT image slice of oak (*Q. robur*) is shown, with both earlywood and latewood
622 vessels segmented.

623
624 **FIGURE AND TABLE LEGENDS:**
625 **Figure 1: General methodological decision tree for X-ray CT scanning.** The rows indicate the
626 steps to take, starting from the research goal all the way to the final data format. White boxes
627 are the steps that are relevant for this toolchain. Greyed-out boxes are steps that can be
628 performed with other software or R packages, such as dplr⁴⁷ and Treeclim⁴⁸ for tree-ring analysis,
629 and ROXAS⁴⁴ as well as ImageJ⁴² or other (commercial) applications for deriving wood anatomical
630 parameters based on the CT images.

631
632 **Figure 2. The HECTOR scanning system.** The system³⁵, showing the source detector distance
633 (SDD) and the source object distance (SOD).

634
635 **Figure 3. Scanning set-up examples.** (A) A log, (B) a cello⁴⁹, (C) sample holders (type 1) with tree
636 cores for batch scanning and (D) sample holder type 2 with increment cores for helical scanning
637 mounted on the rotation stage of HECTOR.

638
639 **Figure 4. Workflow for extracting and drying cores prior to scanning.** (A) Increment cores in
640 paper straws are first put in a hot water bath and subsequently in a hot ethanol-toluene mixture
641 with a Soxhlet apparatus for 24 h then dried, put in a hot water bath for 24 h, then dried again at
642 103.5 °C, then conditioned before scanning. (B) Images of the adapted Soxhlet set-up at Ghent
643 University. Multiple adapted Soxhlet apparatuses are serially attached. Note the aluminum foil
644 and insulation tubes to keep the solvent or solvent mixture warm enough for extraction. (C)
645 Example of the surface of an increment core from *Pinus longaeva* before and after extraction.
646 Resins and other extractives mask the true density signal, due to attenuation of the X-rays.

647
648 **Figure 5. Sample holder types and resolution.** Design of the three main holder types, that
649 correspond to (A) inter-ring scale, (B) tree ring scale, and (C) anatomical scale. Due to the
650 resulting size, the number of samples decreases with increasing resolution requirement. The
651 corresponding 3D renderings for (D) inter-ring scale (holder 1), (E) ring width and MXD (holder
652 type 2), and (F) anatomical parameters (holder type 3). Scale bar = 5 mm.

653
654 **Figure 6. Obtaining wood density values from grey values.** Conversion of the grey values of the
655 image to actual wood density estimates, through calibration with a reference material. (A)

656 Sample holder 1, at 60 μm resolution, showing the air reference, white reference, and the core.
657 (B) Sample holder, at 20 μm , air, reference and core are shown as well.

658

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

663

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

669

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

672

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

679

680 **Figure 11. Screenshot of the CoreComparison module.** Screenshot of the CoreComparison
681 module, where ring width series are distributed vertically for easy visual comparison. The top
682 pane shows the ring width of 4 cores from *Fagus sylvatica*, the bottom left pane shows the
683 Gleichläufigkeit (GLK) and the bottom right pane the Spearman correlation coefficient.

684

685 [Place Figure 12 here]

686

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

694

695 [Place Figure 13 here]

696

697 **Figure 13. Chronology development.** A minimum density and a maximum latewood density
698 chronology from *Widdringtonia cedarbergensis*. Data extracted from²⁶. The scanning resolution
699 is 10 μm .

700

701 [Place **Figure 14** here]

702

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

708

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

712

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

715

716 **DISCUSSION:**

717 Critical steps within the protocol

718 Critical steps within the protocol include proper handling of the increment borer to obtain high-
719 quality increment cores (step 1.1. and see³⁹) to avoid bits and pieces. Next, it is essential that
720 cores be left unmounted (but see²⁶), both for insertion in the sample holder (**Figure 5**, see²¹) as
721 well as for proper resin extraction⁵⁰ and for possible future analysis. Right before scanning (step
722 2.2.2.), the conditioning of the samples in the scanner room is essential to avoid dimensional
723 deformations due to fluctuations of moisture. Then, once the rings can be indicated in the
724 RingIndicator module, it is essential that the ring boundaries and angles are well indicated,
725 because the density peaks can flatten, similar to inaccurate indications on classic densitometry
726 systems¹³. The indication is also important to have correct ring width measurements²¹. The next
727 critical step is proper cross dating through the CoreComparison module (step 4.4.1.). If some
728 cores have wrong indications, there could be an environmental signal loss⁵¹.

729

730 Modifications and troubleshooting of the technique

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

736

737 Limitations of the technique

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

744
745 Significance with respect to existing methods
746 Using the X-ray CT toolchain for increment cores presented here has been tested as showing
747 reliable density values due to the calibration step (**Figure 6**)⁵⁶ and generates MXD values
748 comparable to existing densitometry procedures¹³.

749
750 Future applications of the technique
751 Any future applications of the technique might include the application of deep learning, amongst
752 others for the automated ring indications and wood tissue quantifications⁵⁷, specifically for 3D
753 images.

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763 The authors have no conflicts of interest to disclose.

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787

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