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Note technique sur la mesure des cernes d'arbres à l'aide de CoreTom microtomodensitomètre

A technical note on the tree-ring measurement using CoreTom μ-CT

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Résumé

Ce rapport présente un protocole adapté au système CoreTom microtomodensitomètre de l'Institut national de la recherche scientifique (INRS) pour mesurer la largeur des cernes à partir de carottes de bois dans le cadre d'une collaboration avec le Ministère des Forêts, de la Faune et des Parcs (MFFP). Le protocole comprend trois étapes principales : la préparation des échantillons (section 2), la numérisation des échantillons (section 3) et la mesure des cernes des arbres (section 4). Dans la section 5 (informations supplémentaires), plusieurs réglages de scanner et logiciels de mesure des cernes testés sont décrits.

Abstract

This report introduces a protocol that is suited to the CoreTom µ-CT system of Institut national de la recherche scientifique (INRS) to measure tree-ring width from wood cores under a collaborative framework with Ministère des Forêts, de la Faune et des Parcs (MFFP). The protocol consists of three major steps: sample preparation (Section 2), sample scanning (Section 3), and tree-ring measurement (Section 4). In Section 5 (supplementary information), several tested scanner settings and tree-ring measurement software are outlined.

1. **Required materials and software**
   a) Samples: 5 mm diameter wood cores.
   b) Sample holders: 8 mm paper straws for sample storage (Figure 1) and a custom-made sample holder that accommodates paper straws (Figure 2).
   c) Software: Acquila and Panthera software provided by TESCAN are needed to obtain 3D images of wood core samples. A Matlab-based toolchain box (De Mil et al., 2016; Jan van den Bulcke et al., 2019), including DensitometryToolbox, DHXCT, and CoreComparison software, are used to extract individual wood cores from 3D scans and to measure tree-ring data (ring width and density). To run these Matlab-based programs one needs to install Matlab Runtime v2021b, that is free of charge. However, no knowledge of Matlab is required to use this toolchain box.

In addition to DHXCT, several software are available to measure tree-ring data, such as the XRing R package (Campelo et al., 2019), WinDendro (Regent Instruments Inc.), and CooRecorder (Cybis Dendrochronology). We compared the measurements obtained using the different software in Section 5.

2. **Sample preparation**

In this step, 5 mm diameter wood cores are placed in 8 mm paper straws (Figure 1). Sample ID needs to be labelled on straws using both ink pen and pencil.

2.1 Closing one side of straws using staples

One side of the straw is closed using a staple (Figure 1), while the other side is temporarily folded to avoid sample movement. The use of staples to close both sides MUST be avoided because metals could lead to strong beam hardening image artifacts. The bark side (outer part of a wood core) should be near the side where the staple is used in all cases.

2.2 Ethanol extraction

The non-destructive ethanol (90% ethanol) extraction is needed to pre-treat samples so resins and other non-structural substances (e.g., tannins) could be removed. This step is critical even for ring-width measurement because non-structural substances will heavily blur tree-ring boundaries according to our tests (see Section 5). Extraction of multiple cores (filled in straws) takes place in a Soxhlet extractor and lasts for 48 hours. It is recommended to place the folded sides of straws upward to facilitate extraction. After extraction, samples should be air dried in chemical hood with ventilation on for 5 days.

2.3 Closing the other side of straws using Scotch tapes

After air-drying, the other side of straws can be closed using Scotch tapes (Figure 1).

2.4 Additional notes

Storing cores in fluting papers is not ideal as we've found that many cores broke into pieces in the fluting paper, likely due to a smaller diameter of the holes than wood cores.

Figure 1. Samples stored in paper straws.

3. Sampling scanning

Fast scans using the CoreTom CT scanner and the associated software (Acquila and Panthera) are producing clear images of tree-rings with a resolution of 25 µm. Better image qualities can be obtained by using different scan settings (see Section 5.1 for details). Users could also opt to use higher or lower resolutions (15 and 35 µm) with the current sample holder, but operating times will vary significantly (Table 1).

Materials required: above prepared samples, a 9-hole sample holder that accommodates straws (Figure 2).

3.1 Image acquisition

Samples are filled in a custom-made sample holder, and the holder is placed on the rotating plate of the µCT scanner (Figure 2). The sample IDs and corresponding number of holes need to be recorded on an Excel sheet. Operating parameters are then set up for the scans (the recommended set up is medium quality 2, MQ2 in Table 1) and the STAMINA scan mode is used to scan long samples along the vertical direction. The currently recommended parameters allow for a rapid scan of nine wood cores with a resolution of 25 µm (average from one slice), which takes about 23 min to scan nine 15 cm long samples. Standard scan settings for other resolutions and different image qualities are listed in Table 1. Users should choose proper parameters from Table 1 according to research interests.

Table 1. Standard operating parameters for acquisition CT images of 9 wood cores.

Parameters	Medium-quality scan			High-quality scan		
	15µm (MQ1)	25µm (MQ2)	35µm (MQ3)	15µm (HQ1)	25µm (HQ2)	35µm (HQ3)
Voltage (Kv)	80	80	80	80	80	80
Camera mode	HW1SW1LOW	HW1SW1LOW	HW1SW1LOW	HW1SW1LOW	HW1SW1LOW	HW1SW1LOW
X-ray filter	No	No	No	No	No	No
Exposure time (ms)	100	100	100	270	230	120
No. averaged slices	1	1	1	3	3	3
Voxel size (µm)	15	25	35	15	25	35
No. projection	Default	Default	Default	Default	Default	Default
Unit time est. (min)	6.5	5	3.5	40.5	30	12
No. of sub-scan	8	5	4	8	5	4
Total time est. (min)	50	25	14	320	150	48

Note: *Unit time est.* is based on the time per sub-scan but may vary according to condition of the scanner. *Total time est.* is the total time needed to scan samples of ~15cm, which is the average size of testing wood cores provided by MFFP. Medium-quality images are good enough for ring-width measurements while high-quality images are likely more useful for density measurements. A resolution of 35 µm is too low for measurement of broadleaf (diffuse-porous) tree species.

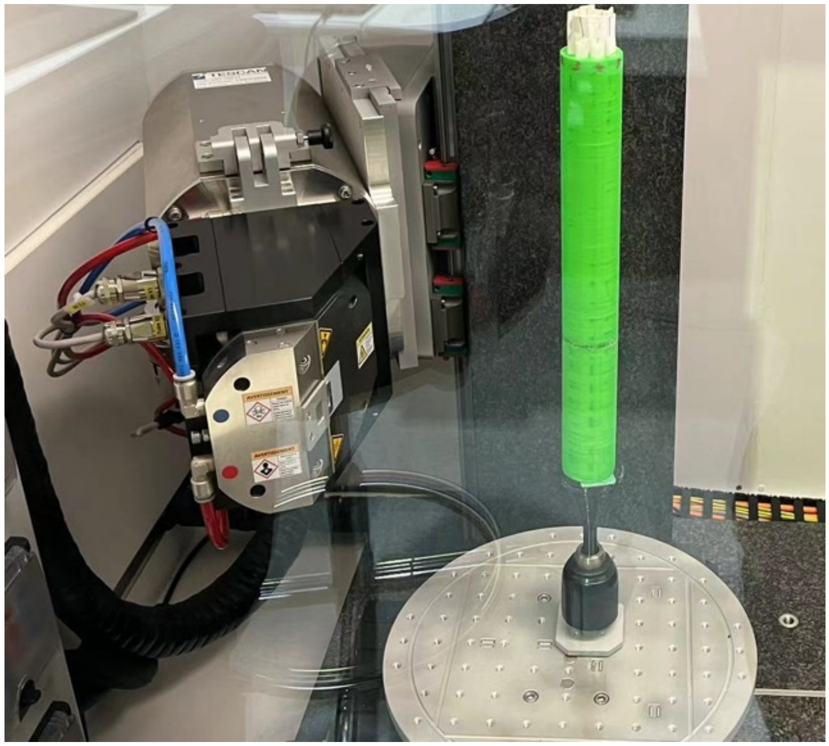

Figure 2. Sample holder on the rotating plate. Samples are filled in the green sample holder.

3.2 Image reconstruction

In this step, acquired images are reconstructed to form a 3D image of the scanned holder along with filled wood cores (Figure 3). Reconstruction settings in the CoreTom Panthera software are adjusted to obtain optimal image quality: beam hardening correction coefficient (BHC coeff) = 0.2, noise filter = 1. A stepwise-based blending mode must be selected on the output menu. It takes 20 min, 60 min, and 120 min to reconstruct one 35-µm, 25-µm, and 15-µm scan of 15 cm samples, respectively. Multiple reconstructions can be batched to run overnight.

3.3 Additional notes

Nine samples could be filled in one sample holder for measuring ring-width. Several sample holders could also be stacked together so an overnight scanning can be performed. The reconstruction settings could be applied to a group of scans.

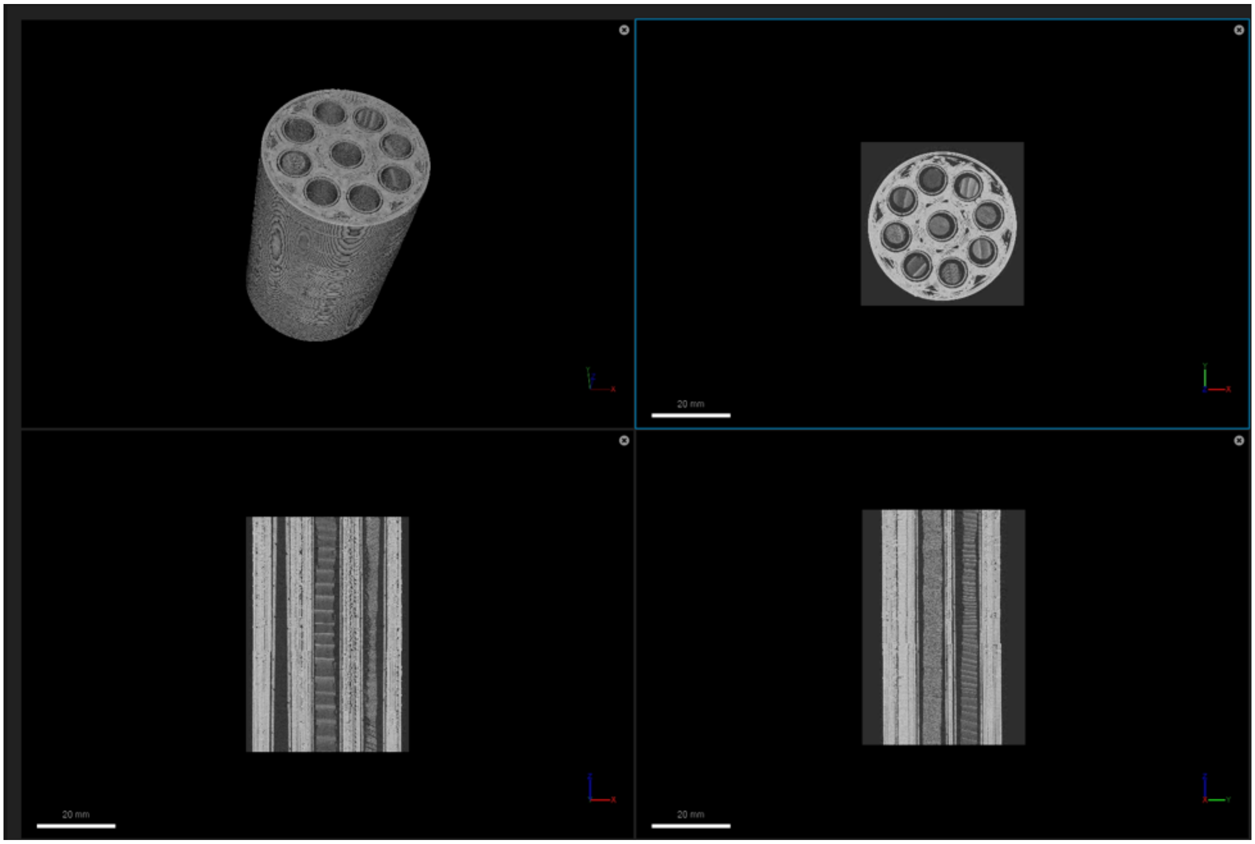

Figure 3. An example of one reconstructed 3D image. Wood cores filled in the sample holder can be easily seen.

4. Tree-ring measurement

For this step, one uses the reconstructed images, several Matlab-based software including DensitometryToolbox, DHXCT, and CoreComparison software (De Mil et al., 2016; Jan van den Bulcke et al., 2019).

4.1 Extracting core images

Using the DensitometryToolbox (De Mil et al., 2016; Jan van den Bulcke et al., 2019), reconstructed 3D images could be processed and individual core samples could be extracted according to user's definition (Figure 4). In this step, a kind of density calibration is mandatory even for obtaining ring-width data because the software assumes that density will also be measured at the same time. If the objective is to measure ring width only, meaning 9 samples are filled in the holder, the holder itself can be used as a "reference material" (1.25 g/cm^3). However, the current 3D printed sample holder walls are not homogenous, and this may introduce a small bias. This issue will be fixed if a high-quality sample holder is made for operational purposes. The software also needs to have a "air reference" that can be easily acquired in the space surrounding the holder. In the current DensitometryToolbox version, the real resolution of output images is seemingly missing, but we have developed some Matlab scripts to further process the images (see section 5.2).

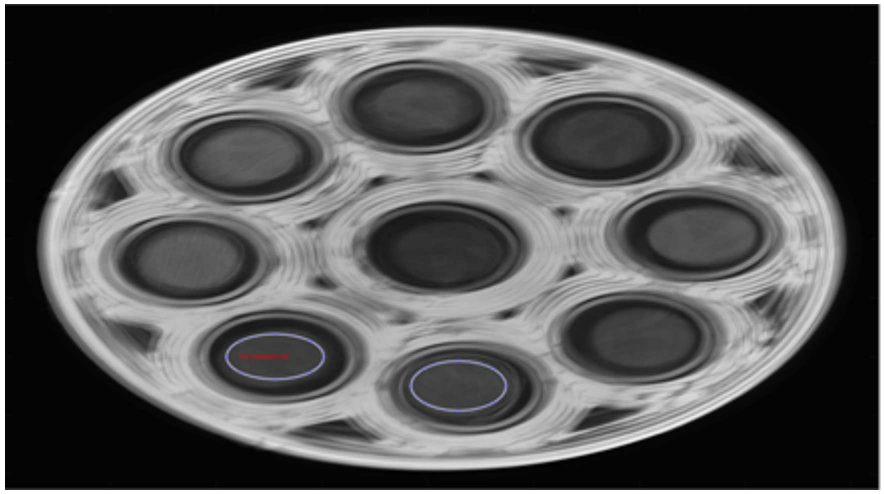

Figure 4. Extraction of wood cores from a 3D scan of 9 wood cores. Note blurry view is normal because the DensitometryToolbox displays a stacked average across the height. The blue circles indicate cores to be extracted. One needs to specify sample IDs orderly in the Excel sheet provided along with the DensitometryToolbox.

4.2 Measure tree-ring parameters

Basically, the extracted core 3D-images can then be imported into the DHXCT software (De Mil et al., 2016; Jan van den Bulcke et al., 2019). The DHXCT automatically detects ring boundaries within a 3D volume (both along cross and longitudinal directions); but sometimes positions and angles of some tree rings are not precisely detected. Thus, a manual correction could be further used to correct wrongly, automatically detected rings (see corrected boundaries in Figure 5a). After defining positions and angles of desired rings, a grain angle correction can be applied so that 3D images will be converted to a stack of cross sections of wood cores.

The major disadvantage of this software is that it does not take into account of curved rings near the pith, i.e., it assumes that all rings are straight along a direction. As a consequence, ring width of very curved rings are not precisely / cannot be measured. The authors of the DHXCT claimed that this is not a big issue for dendroclimatic studies because some juvenile rings near the pith are usually discarded.

4.3 Further data processing

The CoreComparison software could be used to export tree-ring data from the output of the DHXCT. And exported data include ring width, maximum, minimum, and mean tree-ring density. Although CoreComparison also allows for cross-dating, this function does not seem user-friendly. We suggest cross-dating tree-ring data using some other dendrochronological tools, such as PAST5, dplR R package, COFECHA.

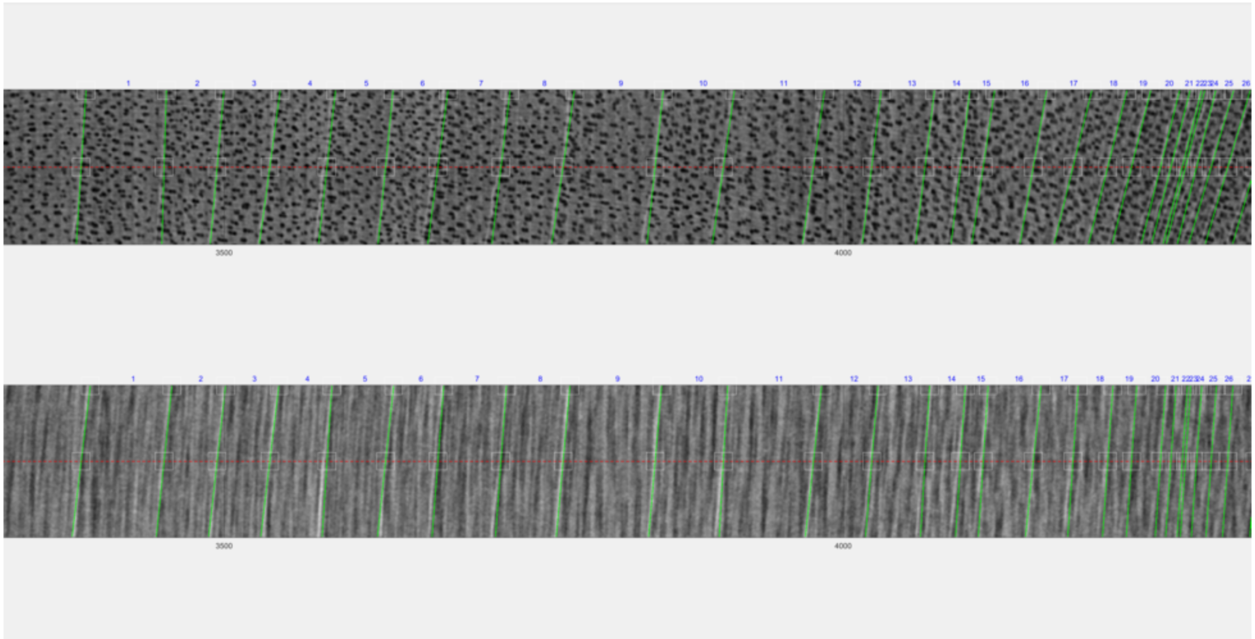

Figure 5. Tree-ring measurements using the DHXCT software.

5. Supplementary information

5.1 Different scan settings and image quality

Although tree rings are clearly seen using the above protocol (MQ2 in Table 1), which aims to obtain a time- and cost-efficient way to measure tree-ring data using the advanced CT technology, better image quality and different resolutions (15 and 35 µm) are possible depending on user's needs. For example, one may expect scans with less noises especially for precise density measurements. Here, we compared processing time and image quality (assessed as variance of gray values in the blank, air target relative to the range of gray values of the entire scan) of different scan settings for three target resolutions, i.e., 15, 25, and 35 µm, using two wood cores per species (black spruce, balsam fir, birch, and poplar). Operating parameters (voltage, exposure time, etc.) of 15 and 35 µm scans were set the same as those used for the two tests for the 25 µm scan (Table 1) for a more direct comparison. Images of four tested species produced using different scan settings are provided as example images.

In general, all the above settings for three resolutions are sufficient to provide very clear images where tree-ring boundaries of test samples provided by MFFP are well distinguished (example images), in particular for coniferous species (black spruce and balsam fir). This means that tree-ring width data can be measured from almost any scan. Nevertheless, tree-ring boundaries of two deciduous species (birch and poplar) are indeed blurrier in the case of 35 µm because tree-ring transition zone (earlywood to latewood) is very narrow (not a matter of image quality). Thus, we recommend a minimum resolution of 25 µm when one wants to measure ring width of diffuse-porous wood samples.

Images of lower resolutions tend to show less noises than those of higher resolutions given the same scan settings (Table 2). Under the same resolution, a greater number of averaged slices and a larger exposure time produce a better image quality (when number of averaged slices is set more than 3, the improvement is less evident). However, the total time for image acquisition and subsequent reconstruction increases significantly (Table 2). One should also notice that exposure time should not be overset, otherwise images will be overexposed (see example for 35 μm-230ms-1avg in Table 2 and example images). In practical operation, the optimal exposure time should be set according to Table 1 so that image profiles of blank background (i.e., air) does not exceed the 48000 intensity value in the Acquila software. The use of three averaged slices and exposure time in Table 1 – "High-quality scan" is deemed as the operating parameters to obtain optimal, high-quality scans, with a compromise of processing time. Again, an exposure time of 100 ms with settings in Table1 is also capable of producing clear images enough for tree-ring width measurement. In addition, one should notice that scans of different resolutions require different space in the disk (Table 2).

Table 2. Comparison of different testing scans.

Parameters	Res. (μm)	Test1 100ms-1avg-LOW	Test 2 100ms-3avg-LOW	Test 3 100ms-5avg-LOW	Test4 230ms-1avg-LOW	Test5 230ms-3avg-LOW	Test6 500ms-1avg-HIGH
Total time of scan (min)	15	50	125	240	100	270	210
	25	25	64	105	50	150	108
	35	14	39	60	28	NA	60
Reconstruction time (min)	15	120	120	120	120	120	120
	25	60	60	60	60	60	60
	35	20	20	20	20	NA	20
Scan + 3D image size (GB)	15	160+85	160+85	160+85	160+85	160+85	160+85
	25	70+37	70+37	70+37	70+37	70+37	70+37
	35	33+15	33+15	33+15	33+15	NA	33+15
Background noise* (mean ± SD)	15	0.079±0.003	0.066±0.001	0.062±0.001	0.064±0.003	**0.054±0.001**	0.061±0.004
	25	0.066±0.003	0.041±0.003	0.043±0.004	0.045±0.002	**0.032±0.005**	0.032±0.006
	35	0.046±0.003	0.033±0.002	0.029±0.002	0.034±0.003	NA	0**.031±0.002**

*: background noise is estimated based on the variance of gray values in the air region relative to the total range of gray values for each slice. 10 random slices are used to provide mean and standard deviation (SD) estimates. Other operating parameters for Tests1–6 can be found in Table 1. Scan settings producing smallest noise are in bold. The 35 μm Test5 was not tested as an exposure time of 230 ms seemed to slightly overexpose the scan (NA data).

5.2 Comparison of tree-ring measurement software

As the DHXCT software may produce biases for curved rings, one could select one 2D image with the best quality from the wood volume and use other commercial or open-source software to measure tree-ring data, such as WinDendro that is currently used by MFFP for the forestry inventories. According to our experience, XRing R package (Campelo et al., 2019), WinDendro, and CooRecorder allow for measuring tree-ring data efficiently. Here, we summarize characteristics of the four software (Table 3) and compare ring width and density (maximum latewood density, MXD) measurements of some selected samples.

Table 3. Characteristics of tree-ring measurement software.

Software	Advantage	Disadvantage
DHXCT	Free; automated ring detection; measurement from 3D images.	Biases in curved rings; ring detection is insensitive (time consuming to manually correct for detection); width measurements need to scale according to size of pixels (maybe improved in the next version).
XRing	Free; automated ring detection (very precise for conifers); measurement from 2D images.	Cannot zoom in images when drawing measurement paths; tree-ring delimitation is based on density profile, rather than the real image, thus it is difficult to measure diffuse-porous wood samples; width measurements need to scale according to size of pixels; multiple paths perpendicular to rings are generally required for measuring one entire core sample.
WinDendro	Automated ring detection; measurement from 2D images; ring boundaries can be oriented for more precise measurements.	Expensive; the current licence owned by MFFP only reads 8-bit images. It means that 16-bit tiff files (where density values were saved in 0.001 g/cm^3) have to be rescaled to be readable; this conversion of gray values is time-consuming. Note that a 16-bit licence of WinDendro exists.
CooRecorder	Cheap; automated ring detection; measurement from 2D images.	Density data are obtained from pre-set windows rather than continuous density profiles; thus one can only obtain percentile of density data (e.g., the 10% highest density) instead of real maximum density data; density windows are not perfectly placed for narrow rings; the 16-bit tiff images will be automatically converted in the software, conversion of gray values is very complicated.

5.2.1 Ring-width data

It showed that all the four software are capable of producing very similar ring-width data (Figure 6). Thus, users can choose any software to measure ring-width data according to their experience and familiarity. But note that very earlier rings cannot be measured by the current version of the DHXCT software.

We developed some Matlab scripts to ensure that the images delivered to MFFP have the following characteristics for an easy use with the current WinDendro Licence: wider blank image frames than wood cores that allow placing measurement paths outside the scanned samples; oriented the wood core images with the young rings to the bottom; images in TIFF format with correct DPI value written in image details according to actual pixel size of the scans (i.e., 25 μm).

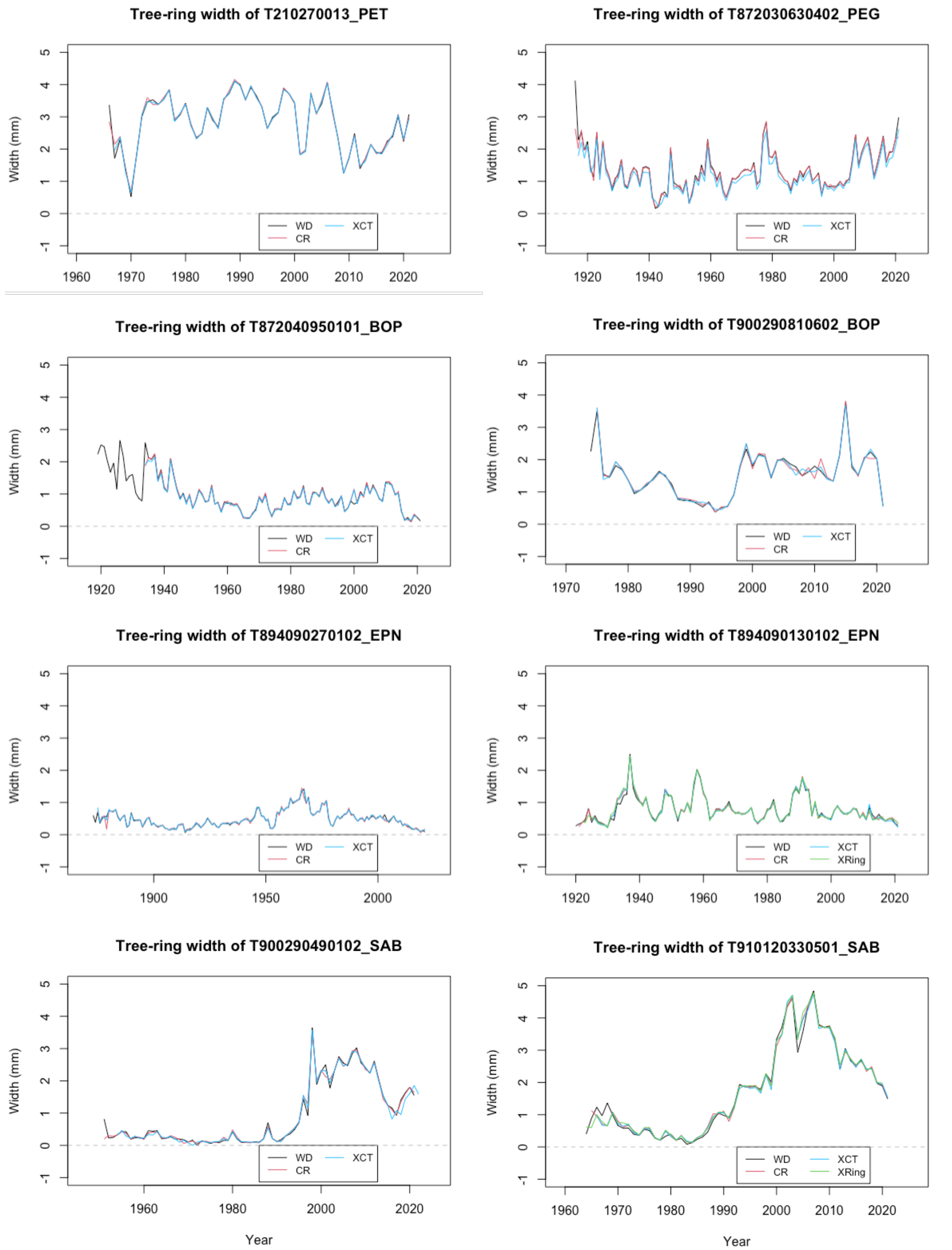

Figure 6. Comparison of ring-width measurements of eight selected samples. WD: WinDendro; CR: CooRecorder; XCT: DHXCT; XRing: XRing R package. Note: measurements of DHXCT are generally shorter than those of WD because of the potential "curved ring" issue discussed above.

5.2.2 Density data

In contrast to ring-width measurement, production of density data appears to be challenging (Table 3). Measurements using different software (DHXCT was not tested since there are some bugs to be fixed) might vary significantly. For example, using CooRecorder and XRing, MXD of sample (ID: 894090130102, black spruce) is highly correlated (r = 0.78) while MXD of the sample 910120330501 (balsam fir) is very different (r = 0.23) (see a comparison in Figure 7). The XRing likely generates more accurate density data because the measurement is based on density profile (CooRecorder is based on 10% darkest pixels in each measurement window, data are thus constrained by the ring width).

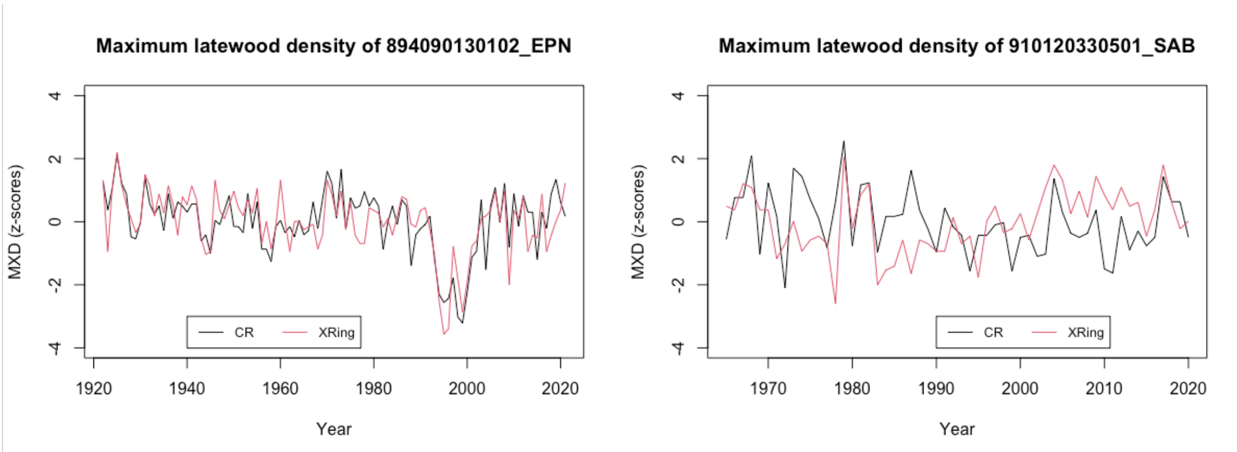

Figure 7. Comparison of maximum latewood density measured using CooRecorder (CR) and XRing for two selected coniferous species. Note: time series are z-scored due to the unknown value conversion in CR.

5.3 Effect of resin extraction

It has also been noted that some portions of scanned wood cores are blurry and tree-ring boundaries are not clearly recognized (Figure 8). This is very likely due to resin or some other non-structure substances remaining in wood samples because their densities are very similar to cell wall (e.g., density of resin is about 1 g/cm^3). We tested this hypothesis by treating four wood cores (one per species) using 90% ethanol. Wood cores along with paper straws were simply immersed in 1L ethanol in a sealed plastic container, and the container was placed on a shaker (at low speed) for 96 hours. This simple extraction approach is still much less efficient than a Soxhlet extractor. Blurry rings disappeared after ethanol treatment (Figure 8), indicating that resin extraction is necessary and likely mandatory even if one only focuses on ring-width measurement. This approach would also definitely allow for production of density data, as an additional benefit.

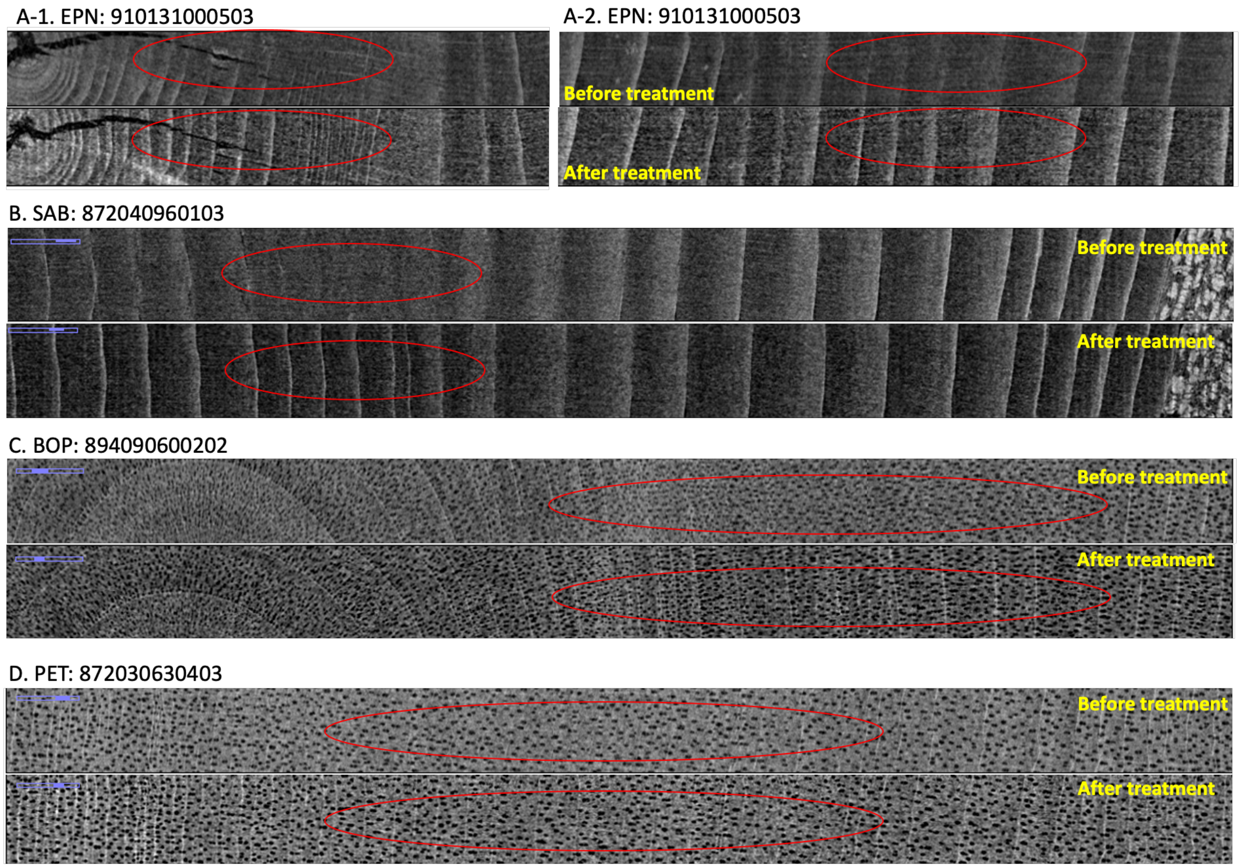

Figure 8. Effect of resin extraction (90% ethanol treatment) on the clearness of tree-ring boundaries.

6. Concluding remarks

In this project, we show the great potential of the CoreTom micro-CT scanner in tree-ring research. We develop a protocol with wide range of options for non-destructive visualization of wood cores, including various qualities and resolutions. This protocol avoids conventional labour-intensive procedures, and can further facilitate ring-width measurement of tree cores collected by MFFP. Tree-ring width data can be easily and precisely measured using several software. In contrast, measurement of density data remains still challenging, yet feasible, given that most current open-source softwares have their own limitations regarding density measurement. Thus, development of a powerful and efficient open-source software for density measurement is critically needed in the future. Improving the DHXCT program is promising with future collaboration with Jan van den Bulcke and Tom De Mil.

Acknowledgement:

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