Fibre directions at a branch-stem junction in Norway spruce: a microscale investigation using X-ray computed tomography

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Abstract

The connection between branch and trunk in a tree must be strong enough to transfer all loads acting on the branch, and it is well known that such branch-stem connections are indeed very strong. In this paper, X-ray computer tomography is employed to investigate the local fibre orientation in the close surrounding of a knot in a Norway spruce specimen to better understand the origins of the mechanical strength of the branch-trunk connection. First, a wood specimen containing an entire knot from pith to bark was imaged with a voxel size of 52 μm . Subsequently, smaller specimens were cut from this original specimen and imaged again with increasingly higher resolution over four levels. With the highest resolution level (2.6 μm voxel size), the tracheids with smallest lumen were successfully traced. The results revealed how the direction of the fibre paths that start below the knot curve around it as the paths progress upwards to the region just above the knot, where the paths divide into two: one set of paths integrating with the knot on its top side and the other set continuing up along the trunk. Fibres that integrate with the knot at its top follow paths just before they continue into the knot, with a radius of curvature of only about 1 mm in both vertical and horizontal directions. No abrupt change of fibre pattern between latewood and earlywood is observed; rather, a continuous change of fibre direction across annual layers can be seen. The detailed characterisation of the local fibre structure around the knot provides new data that can explain the remarkable strength of the branch-trunk connection.

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Introduction

The integration between branch and stem in a tree must be strong enough at the junction to transfer all loads acting on the branch to the stem, i.e. the self-weight of the branch and loads related to snow and wind, but also to enable transport of nutrients and water between stem and branch. Wood is strong and stiff in the direction of fibre, i.e. the tracheids, which are the narrow tube-like cells that constitute 90–95% of clear wood of softwood (e.g. Hoffmeyer 1995). In directions perpendicular to the tracheids, however, strength and stiffness are much lower. Therefore, wood is generally regarded as an orthotropic material. In Norway spruce clear wood, tracheid direction almost coincides with the longitudinal direction of the tree trunk, but with a small angle in the longitudinal-tangential plane related to so-called spiral grain (Harris 1989; Säll 2002) and in the longitudinal-radial plane due to taper of the stem. The microstructure and dimension of clear wood tracheids are well known since extensive research has been performed on clear wood (e.g. Lundqvist et al. 2018; Piermattei et al. 2020). However, except that tracheids in Norway spruce knots are smaller than tracheids in clear wood and that the knots have higher density compared to clear wood, little is known about wood and tracheid properties in the branch and in the transition zone where the branch connects to the trunk. Regarding terminology, the two terms *tracheid* and *fibre* are herein used interchangeably. This also is the case for the terms *branch* and *knot*.

Eames and MacDaniels (1947) found that the conducting fibres at branch-stem junctions are arranged in distinctive patterns and that the cell structure of these fibres is very different from that of clear wood. Lev-Yadun and Aloni (1990) carried out a microscopy study on samples of wood at the branch-stem junctions of 15 species including both hardwoods and softwoods; their study showed that curved tracheid and circular vessels normally occur in branch junctions. Curved and circular tracheids can restrict water conductivity through branch junctions, such that supply of water to other branches, further up the stem, will be sufficient. Müller et al. (2006) studied the biomechanics of the branch-stem junction by determining the strain field in a mechanically loaded Norway spruce branch-stem junction using electronic speckle pattern interferometry (ESPI). To determine strains in a vertical plane of a sound branch-stem junction cut from a living Norway spruce tree, the junction was split into halves through both the pith of the stem and the pith of the branch. One half of the junction was loaded by a downward directed force on the branch at a certain distance to the stem. In-plane strains were determined by application of ESPI over the surface in which both the pith of the stem and the pith of the branch were visible. The results showed a homogenous distribution of strains in both vertical and horizontal direction, without any large local concentration of strains anywhere in the branch-stem junction. This implies that fibres in the junction are oriented such that large stresses in directions perpendicular to the local fibre direction are effectively avoided. Moreover, branches and junctions must also provide damping to dynamic oscillation of the tree. Dynamics of trees and branches have been studied, for example by James et al. (2006), Spatz et al. (2007) and James (2014).

An early model explaining the local fibre orientation at a branch-stem junction was presented by Shigo (1985). This model is based on the hypothesis that branch tissue and trunk tissue, respectively, develop at different periods within every growth season. In this context, the term *tissue* refers to a group of fibres of similar makeup. According to Shigo, the branch tissue would start to develop before the trunk tissue early in a growing season. The branch fibres, which are oriented along the stem below the branch, would encircle the branch and form a collar around it before they extend into the branch. Trunk tissue would develop later in the growing season and pass around the branch without integrating with it. Shigo's model was established based on dissection, debarking and conduction experiments using dyes plus microscopic observations on large numbers of branch-stem junctions of a wide range of tree species. This model has often been regarded as credible by other researchers investigating the attachment to the trunk. However, Shigo's hypothesis and explanations have also been questioned. For example, Nelly (1991) studied how sap flows at branch junctions using conduction tests with dyes. In this study, water-soluble dye was injected into stems through pre-drilled holes beneath branches at various time over a growth season. Thereafter, the stems and the branches were peeled off and the uncovered surfaces were examined to trace the dye translocations. The results showed that patterns of dye movement did not vary with the time of injection, which did not support Shigo's hypothesis regarding the varying patterns of branch and trunk fibres over each growth season. However, in Nelly's study, the pre-drilled holes for the dye injection were 1 mm in diameter and 5 mm deep. Dye in such big and deep holes could penetrate both the branch tissues and trunk tissues described in Shigo's model, making it difficult to observe any possible variation in patterns over a growth season. Moreover, the conduction tests with dyes, both by Shigo (1985) and by Nelly (1991), show that sap flows from the trunk to the branch only from below the branch, not from above it. On the basis of this observation, Shigo (1986) concluded that the trunk tissue from above the branch does not connect with the branch. This conclusion was, however, questioned by Slater and Harbinson (2010); they argued that the branch and stem are connected at the top by other types of cells, which do not conduct fluids but can provide mechanical support.

Recent microscopy studies on the softwood branch junction (Müller et al. 2015, 2018) have shown that tracheids form apparently complex interwoven patterns in a region just above the branch. Such a region is marked in Fig. 1a and enlarged in Fig. 1b. How the fibres are actually oriented in this region is, however, partly incomprehensible when only studying two-dimensional (2D) microscopy images. A proper understanding of the fibre patterns in the junction requires high-resolution 3D imaging and visualization, for example, by X-ray computer tomography scanning, with sufficient resolution.

X-ray computed tomography (CT) is a state-of-the-art technology in non-destructive analysis of various materials. CT consists of two parts, namely the X-ray image acquisition and computing tomographic images. When acquiring the data, the X-ray beams pass through the object and attenuation of the X-ray radiation occurs through absorption or scattering. An X-ray detector records the transmitted beam resulting in a 2D image that shows how much of the incident X-ray beam penetrated the object at different positions. In a CT device, either the X-ray source and the detector both

Fig. 1 Microscopic image of a wooden slice cut from a branch-stem junction of Norway spruce, including branch wood, showing two complete annual growth layers inside the knot and the surrounding wood tissues. An area above the knot is highlighted in **a** and an enlarged view is shown in **b**

rotate around a fixed object or the object is rotated while the source and detector are fixed. In both cases, multiple transmission images (radiographs or projections) are obtained from different angles around the object. Based on these images, the second part of CT is to compute 3D representation of the object. A review of the technique for material science is given in Withers et al. (2021).

CT was first introduced for application within medicine in the early 1970s and soon proved to be very useful; it is now indispensable in radiological diagnosis (e.g. Kalender 2011). The application of CT in wood science and technology started in the 1980s, when only medical CT scanners were available. Such devices were used for scanning of logs to detect pith, decay and sapwood, as well as annual rings, knots, etc. (e.g. Benson-Cooper et al. 1982; Grundberg 1994; Oja 2000; Seifert et al. 2010; Nikolova et al. Nikolova et al. 2009; Fredriksson 2014). Interpolation methods were also developed to longitudinally interpolate between consecutive tomographic scans along the stem axis (zu Castell et al. 2005). Subsequently, research and development work to apply CT in sawmills (e.g. Taylor et al. 1984; Funt and Bryant 1987; Nordmark 2005; Giudiceandrea et al. 2011) led to the launch of the first high-speed industrial log CT scanner in 2011. Today, CT scans are performed on logs in production lines of sawmills to identify the internal features for grading logs and for optimizing the orientation and how to saw each log (e.g. Rais et al. 2017).

Typical resolution for an industrial log and timber CT scanner is about 1 mm in transversal direction and 10 mm in longitudinal direction. However, detection of individual fibres in the vicinity of knots requires a high-resolution micro-CT scanner. Using micro-CT, a wood volume can be imaged and reconstructed with a voxel dimension of about 1/2000 in relation to the size of the specimen. Such high resolution enables 3D microscale visualization of the fibres in a wood volume of a few millimetres in size. In the past, such micro-CT has been used to study the anatomy

and vessel arrangement of hardwood forks (Slater et al. 2014), which showed a complicated tortuous vessel pattern in the forks of hazel trees. However, no in-depth studies using micro-CT aimed at visualizing fibre patterns in softwood close to and within knots are known to the authors.

The aim of the present research is to provide a 3D understanding of the micro-structure in a softwood branch junction with focus on local fibre orientation. The purpose is to clarify the fibre pattern at the junction and, especially, to elucidate the fibre direction in the contested region above the branch within one or a few annual growth layers. The objectives are as follows:

1. Establish a laboratory method and procedures for using micro-CT to image wood materials cut from a branch-stem junction to obtain tomographic images of target regions;
2. Present dimensional measurements of Norway spruce tracheids in clear wood, branch wood and transition zone wood;
3. Track individual fibres in the micro-CT reconstructed volume by an automatic tracing algorithm and determine the fibre orientation in 3D;
4. Present a 3D illustration of the fibre patterns at the branch-stem junction to, especially, visualize fibre orientation in the region above the branch and within individual growth rings;
5. Contribute to knowledge and understanding of the mechanical connection between trunk and branch in Norway spruce.

Material and specimen preparation

The present study was conducted on Norway spruce (*Picea abies* (L.) H. Karst), from a selected tree that was felled in November 2015 on a stand at the Kråkenäsryd Gårdsby (56°57'17" N, 14°54'06" E) locality in southern Sweden. This stand is well documented. From 1953 to 1962, a series of pre-commercial thinning experiments were established in Sweden, covering a total of 28 localities including 140 stands. The locality and stand from which the tree for the present study was selected was included in these experiments. The tree was planted in the spring of 1962, then a four-year-old plant of provenience Nödebo F71, Denmark. The locality in Kråkenäsryd Gårdsby consisted of nine stands, and the purpose was to study how volume relates to timber quality after thinning to different numbers of remaining stems. The present stand had 1800 stems/hectare after pre-commercial thinning in 2002. Six rectangular wood samples were cut from the selected tree shortly after felling; these samples were all centred around knots with generous margins and had dimensions of about 150×150×500 mm³. The samples were slowly kiln-dried in a laboratory kiln directly after cutting to an average moisture content of about 18%. To avoid further drying and risk of cracks, the samples were thereafter carefully packed with plastic films and stored in a climate room with a constant temperature of 20 °C and relative humidity of 65%. For a detailed description of the cutting procedures and more details of the samples, see Hu et al. (2018).

The wood material for the present study was cut from one of the six samples described above. The selected knot of this sample was located 9.25 m above the ground and was directed southwards in the original tree. This is the only knot studied in this paper. A smaller sample, including the whole knot, was cut from the initial larger sample. This smaller sample was denoted sample 1 (S1) and scanned with a micro-CT with a first resolution level (RL1), before a smaller sample (S2) was cut out from it. S2 was scanned with higher resolution before it was then cut into smaller pieces. This process was repeated to yield a total of four different resolution levels, RL1—RL4. The samples investigated at the different resolution levels were denoted as S1, S2, S3a, S3b, S4a and S4b, where S3a and S3b are two different samples, both cut from S2, and examined on RL3. Finally, S4a was cut from S3a and S4b was cut from S3b.

A circular saw and a band saw were used to cut the samples. The successive reduction in size of the wood samples obtained in the respective steps is illustrated in Fig. 2 with the reconstructed CT volumes marked by red dashed lines. Blue dashed lines are used to mark the part of wood that is identical to the materials shown in the following sub-figures after cutting into smaller pieces. S1 had a dimension of about $75 \times 75 \times 107 \text{ mm}^3$ and included 26 annual rings from pith to bark with an average width of 3.7 mm. S2, shown in Fig. 2b, had a dimension of $32 \times 32 \times 75 \text{ mm}^3$. Similarly, S3a and S3b, shown in Fig. 2c, d, had dimensions of approximately $9.5 \times 9.5 \times 75 \text{ mm}^3$. The annual rings that can be seen on the cross sections of these specimens are ring number 11–15 of the tree. S4a and S4b, shown in Fig. 2e, were about $3.5 \times 3.5 \times 75 \text{ mm}^3$ in size. To prevent drying and to avoid related deformation and cracks, the samples were wrapped in plastic film immediately after cutting and after completion of each scan.

Method

Micro-CT scanning to obtain raw data

A series of micro-CT scans was performed using a Zeiss Xradia Versa XRM520 (Zeiss, Oberkochen, Germany) at the 4D Imaging Lab at the Division of Solid Mechanics, Lund University. The reconstructed tomography images have a cylindrical field of view (FoV), as illustrated in Fig. 3, allowing 3D imaging of internal structures of the wood material with cubic voxels of side length of about 1/1000 or 1/2000 of the transverse dimension of the FoV, depending on whether the recorded projection images were binned to give macro-pixels of 2×2 pixels (“binning 2”) or without binning (“binning 1”). Hereafter, the side length of the cubic voxels is referred to as the voxel size of the images.

The CT scans and corresponding preparations of samples in this study were carried out in four steps, corresponding to the four resolution levels used, as described in “Material and specimen preparation” section. Thus, RL1 was applied to S1, RL2 on S2, RL3 on S3a and S3b, and RL4 on S4a and S4b. The scans performed on different resolution levels are described in detail below. A summary of the CT scans performed is provided in Table 1.

Fig. 2 Preparation of wood samples for successive CT scans. Red dashed lines mark wood materials that were imaged. Wood marked with blue dotted lines is identical to that shown in the following sub-figure, after being cut into smaller pieces. **a** S1, in which CT scanned volume on RL1 is marked by red dashed lines. **b** S2, marked with red dashed lines. **c** An intermediate cut to prepare for S3a and S3b. **d** S3a and S3b, imaged parts, 50 mm long, marked with red lines and arrows. The parts marked with blue and purple lines were selected for S4a and S4b, respectively. **e** S4a, in which a part about 20 mm long was imaged on RL4

1. RL1: The aim was to image a 3D volume that included clear wood, transition wood and branch wood. The purpose was to establish a reference coordinate system in relation to the knot, by which the volume data from the subsequent scans could be put in context of the larger structure. S1 was imaged with local tomography such that the FoV covered a cylindrical volume of about $\pi \times (52/2)^2 \times 107 \text{ mm}^3$ within S1, as shown in Fig. 2a, with a voxel size of 52 μm (binning 2). To cover the cylindrical volume in longitudinal direction, i.e. 107 mm height, the scanning of S1 consisted of three consecutive sub-scans, for which the sample was translated two times along the vertical direction. The resulting 3D images of the sub-scans were automatically stitched together after tomographic reconstruction. From this RL1 scan, a sub-volume of interest (SVoI) was identified and used to define the preparation of S2, to be scanned with higher resolution, i.e. on RL2.

Fig. 3 Drawing of scan setups showing the X-ray source and the detector. The orange cylinder, just covered by the field of view, represents the investigated part of the wood sample, which rotates around the vertical axis such that X-ray images are taken from different angles

Table 1 Summary of the CT scans performed on four different resolution levels

	No. of scanned samples	Specimen	Sample(s) dim. (mm ³)	Scan FoV (mm ³)	Voxel dim. (μm)	X-ray source (kV/W)
RL1	1	S1	75 × 75 × 107	$\pi \times (52/2)^2 \times 107$	52 (binning 2)	80/7
RL2	1	S2	32 × 32 × 75	32 × 32 × 75	24	80/7
RL3	2	S3a, S3b	9.5 × 9.5 × 75	9.5 × 9.5 × 50	7	60/5
RL4	2	S4a, S4b	3.5 × 3.5 × 75	3.5 × 3.5 × 20	2.6	80/7

- RL2: S2, representing the SVoI identified from scanning of S1, was fully imaged with a voxel size of 24 μm (binning 1). To cover the entire vertical length of S2, two consecutive sub-scans were acquired and stitched, in the same way as for S1 on RL1. Based on the results of this scanning, two new SVoIs were identified and, correspondingly, S3a and S3b were prepared for examination on RL3.
- RL3: S3a and S3b were imaged with a voxel size of 7 μm (binning 1). The middle sections of S3a and S3b in their longitudinal direction, lengths about 50 mm, were each imaged using four consecutive sub-scans that were stitched. Thus, the tomography was local with respect to the longitudinal direction of the specimen but each sub-scan covered the entire specimen with respect to its smaller dimensions. Based on the images, SVoIs inside S3a and S3b, respectively, were identified and, correspondingly, S4a and S4b were prepared for examination on RL4.
- RL4: S4a and S4b were imaged in the same way as s3a and S3b but with a voxel size of 2.6 μm (binning 1) and the scanned volumes were about 20 mm long in longitudinal direction. Five sub-scans were stitched together to cover this length for each sample.

The reconstructed data from the CT scans were output in the form of a series of 2D 16-bit grey-scale images of Tagged Image File Format (TIFF) stacked along the longest dimension of each specimen. For lengths in mm, see ‘Scan FoV’, in column 5 of Table 1. As stated earlier, the voxel was cubic (voxel sizes are given in Table 1), so the pixel size in the plane of the TIFF images is the same as the distance between two consecutive images in the stack; from 52 μm to 2.6 μm depending on the resolution level. The grey-scale intensity in the TIFF images is a relative measure of the spatial distribution of the linear X-ray attenuation coefficient. High grey-scale intensity, represented by light colour in TIFF images, indicates a high attenuation coefficient in the associated region of the sample, which generally corresponds to higher density material.

Volume rendering and visualization of the CT data were performed using the software Avizo (Thermo Fisher Scientific, Waltham, Massachusetts, USA). This software was used to perform post-processing on the 3D imaging data using thresholding, slicing and clipping, to image any selected sub-volume or plane through the scanned volume. Volume renderings were also made to obtain semi-transparent representations by which the selected volumes can be better understood in 3D.

Automatic tracing of fibre directions

In this section, an automatic fibre tracing algorithm, which enables detection of fibrous structures and tracing of fibre centrelines, is described. The two main steps of the algorithm (provided in the extension XFiber of the Avizo software) are *cylinder correlation* and *trace correlation lines*. Using these tools, fibre directions were traced in specimens S3a, S3b, S4a and S4b.

Cylinder correlation

The first step in the fibre tracing was to calculate local cross-correlation of the 3D imaging data obtained on RL3–4, using the *Cylinder Correlation* module of Avizo/XFiber, described in detail in Weber et al. (2012). In this algorithm, the local cross-correlation is calculated over cylindrical sub-volumes centred on each voxel for different orientations of the cylinder, as illustrated in Fig. 4a. The orientation of the cylinder giving the maximum correlation for a given voxel defines the local fibre direction. The output of the cylinder correlation consists of two scalar fields defined at each voxel: the maximum cross-correlation and the corresponding orientation. The cross-correlation results were stored as 8 bits integers of [0 to 255], where 255 represents a perfect match. In this study, the largest cross-correlations obtained were in the range of [225 to 250], depending on the data set and the applied cylinder dimensions.

A cylinder is an appropriate model for the correlation in the current work, as this mimics the central cavity, i.e. the lumen, of a wood fibre in Norway spruce as shown in Fig. 4b. The dimensions of the cylinder are defined by the user and, here, the radius of the cylinder was chosen to be approximately the same as the lumen radius and the length of the cylinder was chosen to be several times the diameter of the

Fig. 4 Illustration of method for identification of fibre direction. **a** A cylinder defined by radius and length to mimic the lumen of a wood tracheid. The cylinder may be orientated in any direction as indicated by dashed lines. **b** Microscopic structure of a piece of clear wood, showing the tubular wood tracheid of both earlywood and latewood. **c** A search cone defined by cone length and opening angle within which the next voxel on the fibre centreline is identified

lumen. Since the size of lumen is different in different parts of the wood samples, settings on the cylinder dimension are crucial to accurately capture the direction of wood fibres locally in the investigated specimens.

Trace centrelines of fibres

The second step in the fibre tracing was line tracing, i.e. tracing the centrelines within fibres, based on the correlation field and the orientation field obtained from the previous step. Detailed descriptions of the tracing algorithm can be found in Weber et al. (2012) and Rigort et al. (2012). In short, a line was started at a voxel, where a direction correlation value was greater than a user-defined threshold, herein denoted the *minimum seed correlation (MSC)*. The tracing algorithm iteratively finds the next voxel on the line by evaluating the probability, based on the information in the correlation and orientation field, for candidate voxels belonging to the same fibre. A candidate voxel is searched within a limited range, namely a *search cone* oriented in the same direction as the tangent direction at the voxel being considered. The line is stopped if the correlation values for all candidate voxels in the search cone were less than another threshold, the *minimum continuation quality (MCQ)*. Two parameters, i.e. *opening angle* of search cone and *direction coefficient (DC)* (varying from zero to one) determine how rapid change of direction, i.e. how small curvature radius, can be traced in the calculation. A larger opening angle enables tracing of lines with smaller curvature radius, but with a higher risk of erroneously identifying spurious curvature because of noise in data. A higher DC has a similar effect to a larger opening angle regarding how small curvature radius can be traced. However, DC is a parameter for fine-tuning. The opening angle is the more important parameter of the two.

Model and visualization parameters

As described above, parameters for cylinder correlation were *cylinder radius* (*Cyl.R*) and *cylinder length* (*Cyl.L*) shown in Fig. 4a. For tracing the centrelines, seven parameters were used, namely *MSC*, *MCQ*, *DC*, the *cone length* (*Cone.L*) and *opening angle* (*Cone.A*) as illustrated in Fig. 4c, and *minimum distance* (*Min.dist.*) and *minimum length* (*Min.L*). *Min.dist.* is the minimum distance, in relation to *Cyl.R*, between two adjacent centrelines. This can be used to avoid one fibre being traced inside another one or to limit the number of fibres to be traced and visualized. *Min.L* was used to eliminate, in the visualization of the results, lines that were shorter than the prescribed value. For the results presented here, *Cone.L*=*Min.L*=*Cyl.L* was used throughout. All combinations of parameter settings used to trace fibres of different sizes and curvatures, as well as to create clear visualizations, are defined in Table 2.

Results and discussion

Visualization of CT data and achieved detail level

Visualizations of CT data for all four resolution levels (RL1–4) are shown in Fig. 5. The figure gives an overview of the reconstructed CT data in terms of 2D slices through the 3D volumes and 3D volume renderings to highlight the level of detail achieved with the different resolution levels. Figure 5a shows results for S1, scanned on RL1, in the form of a combined image where the left part shows a 3D volume rendering of the knot close to the bark and the right part shows a 2D slice image of the part of the knot close to the pith. Note that, only half the reconstructed volume of S1 is displayed (volume clipped through the knot) to reveal the hidden inner structure of the knot. The 3D view (left part of Fig. 5a) was generated using the

Table 2 Combinations of parameter settings used to trace fibres and create clear visualizations

Settings/parameter	Cylinder correlation <i>Cyl.R/Cyl.L</i> (mm/mm)	Tracing centrelines <i>MSC/MCQ/Min.dist.</i> (-/-/mm)
Setting 1	0.003/0.1	210/105/2 × <i>Cyl.R</i>
Setting 2	0.005/0.1	210/105/2 × <i>Cyl.R</i>
Setting 3a	0.007/0.1	210/105/2 × <i>Cyl.R</i>
Setting 3b	0.007/0.1	210/105/20 × <i>Cyl.R</i>
Setting 3c	0.007/0.1	210/105/40 × <i>Cyl.R</i>
Setting 4	0.01/0.1	190/95/2 × <i>Cyl.R</i>
Setting 5	0.013/0.13	200/100/2 × <i>Cyl.R</i>
Setting 6	0.015/ 0.1	180 /90/2 × <i>Cyl.R</i>
Setting 7	0.02/0.1	100/60/2 × <i>Cyl.R</i>

Fig. 5 Visualization of the CT data for all four resolution levels. **a** The reconstructed volume of part of S1 showing 3D volume rendering (left) and a 2D stack image (right). A sub-volume marked with blue lines is also part of S2. **b** The reconstructed volume of S2 and a selected area on RL1 and RL2. Sub-volumes marked with purple and green lines are parts of S3a and S3b, respectively. **c** The reconstructed volume of S3a and S3b and selected area on RL2 and RL3. The sub-volume marked with cyan lines is S4a. **d** Volume of S4a and selected area on RL3 and RL4

Avizo *Volume Rendering* module. A threshold was set for the volume rendering such that only latewood in clear wood and branch wood is visible in this part. In Fig. 5a, a sub-volume is also marked with blue lines. This volume was part of S2 and was thus imaged both on RL1 and RL2. In Fig. 5b, this sub-volume is shown again and a selected small area, marked with red lines, is enlarged to more clearly show the difference between results obtained on RL1 and RL2, respectively. In the 3D images of Fig. 5b, two sub-volumes are marked with purple and green lines, respectively, which correspond to parts (not full lengths) of S3a and S3b, respectively. In Fig. 5c, a reconstructed volume that was part of S3a and S3b is shown. A selected area, at the border of the knot, is enlarged and a comparison shows that the cell structure is distinguishable on RL3 but not on RL2. In Fig. 5c, not only the sub-volumes corresponding to parts of S3a and S3b, but also S4a and S4b are marked, with cyan and orange lines, respectively. In Fig. 5d, the reconstructed volume of S4a (rotated compared to the corresponding volume marked in 5c) is shown. A selected area is enlarged to show the difference between results obtained on RL3 and RL4. As before, most of the cell structure is distinguishable already on RL3, but it becomes much clearer on RL4, where also the smallest cells can be seen clearly.

Tracheid dimension and direction obtained from CT stack images

Figure 6 shows 2D images from the CT data of wood tracheids in regions with clear wood, transition wood and branch wood from scanning of S4a. The left part of the figure shows a rendering of the reconstructed volume of S4a from the pith of the knot in the lower end to clear wood above the knot in the top end. Planes are marked in the rendered volume within sub-volumes in the clear wood zone (a1–2), in the transition wood zone (b) and within the knot (c1–2). In the right part of Fig. 6, CT images at RL4 (voxel size 2.6 μm) are shown for each of the planes marked in the rendered volume in the left part of Fig. 6. All images (a1–2, b and c1–2) are displayed in the same scale to allow for a direct comparison of the size of tracheids in different regions. Image a1 shows an XZ-plane (coordinate system defined in Fig. 6) where the length and width of single fibres in earlywood of clear wood can be seen; a cyan two-headed arrow is used to mark the length of a single fibre. Image a2 shows a XY-plane through the same clear wood sub-volume where cross sections of the fibres in both earlywood and latewood are seen. Image b shows a YZ-plane view of the transition wood zone. Although the displayed surface in image b is only about 0.7 mm in size, it is clear that fibre directions change within this small area. In the top of image b, and in the bottom left of this image, the fibre direction seems to be more or less perpendicular to the displayed plane, whereas fibres in the middle and lower right of the image have another direction. Image b shows earlywood of the

Fig. 6 Tracheid dimensions of different regions. A 3D volume rendering is shown to the left. Three sub-volumes representing clear wood, transition wood and branch wood are marked. The orthogonal views of the sub-volumes are highlighted and shown to the right in highest resolution (RL4), from which dimension of the tracheid can be estimated

transition zone and fibres have, in general, smaller lumen and thicker cell walls compared to earlywood of clear wood. However, the size of the lumina differs considerably between different cells in the transition zone. Images c1 and c2 show longitudinal and perpendicular directions of fibres, respectively, in a region within the knot. Image c1 shows earlywood within the knot, while both earlywood and latewood are seen in image c2. Compared to clear wood, fibres within the knot are shorter and lumen much smaller.

Based on the images on RL4 shown in Fig. 6, lumen size and the length of fibres in different regions were estimated and results are presented in Table 3. Regarding

Table 3 Tracheid dimension and length estimated based on images of S4a on RL4

Region/dimension	Lumen size (μm)	Length (mm)
Clear wood	20–50 (R)	~ 1.2
	15–35 (T)	~ 1.2
Transition zone	10–30	–
Branch wood	5–25 (R)	~ 0.5
	5–20 (T)	~ 0.5

the size of the lumen given in the table, the wide ranges given for radial (*R*) and tangential (*T*) directions occur due to the difference between latewood and earlywood (larger lumen in earlywood than in latewood). No lengths of fibres are given for the transition wood zone, as fibres in that region are curved such that they cannot be measured in a 2D plane.

Figure 7 shows a 3D visualization of a sub-volume from the transition zone, based on scanning of S4a on RL4. The sub-volume highlighted in the reconstructed volume of S4a, of about 1.2 mm width, is enlarged and shown in Fig. 7b, with three displayed sides marked b1, b2 and b3. Side b1 comprises an area similar to what is shown on surface b of Fig. 6, but Fig. 7b also includes the border between latewood (lower 2/3 of b1) and earlywood (upper 1/3 of b1). In Fig. 7c, traced fibres for a volume slightly larger than the displayed sub-volume in Fig. 7b have been added. The traced fibres, represented by coloured curves, are, to the major part, covered by the sub-volume image, but it can be seen how the traced fibres correspond to the cell structure shown on the sub-volume surfaces; a part of side b3 is enlarged to show this clearly. In Fig. 7d, the sub-volume image is removed such that traced fibres within the sub-volume are revealed. The settings 2, 3a, 4, 6 and 7 in Table 2 were used to trace fibres of different size, represented by the coloured curves.

The traced fibres enable a better interpretation of what can be seen on the sides of the visualized volume. For example, in the lower part of the corner between sides b1 and b3, in Fig. 7b, it can be seen that fibres here are directed close to perpendicular to side b1, but with an inclination to the horizontal axis. This agrees with the direction of the traced fibres shown in the lower part of Fig. 7d, in which more details of fibre directions, also within the sub-volume, are revealed. Furthermore, in the highlighted area of Fig. 7b, it can be seen that there are fibres in this part of the sub-volume with quite different orientations, and it becomes clear in Fig. 7d that the fibre direction changes over a very short vertical distance, about 0.5 mm, from an almost vertical direction (blue curves), in the lower part of the highlighted area, to an almost horizontal direction (green/brown curves), to again an almost vertical direction (red/brown curves), in the upper part. However, the transition between latewood and earlywood of the following year (this border is seen at the upper part of the area highlighted in Fig. 7b), which is characterized by a sudden change from small to larger tracheid lumen, does not correspond to any abrupt change of fibre direction at the border itself. In the highlighted area of Fig. 7b, small clusters of smaller cells can be seen. These are rays with a quite different direction than the adjacent tracheids. In Fig. 7d, some traced rays are represented by light blue curves. In conclusion, it is shown that tracheids, and even rays, can be traced

Fig. 7 Visualization of a sub-volume from the transition zone. **a** Reconstructed volume of S4a with sub-volume highlighted, **b** 3D view of enlarged rendered sub-volume. **c** Enlarged rendered sub-volume complemented by traced fibres, represented by coloured curves of a slightly larger volume. **d** Uncovered traced fibres within the sub-volume

in data of RL4, from which the visualization of traced tracheids in 3D provides new detailed information on local fibre direction indicating, for example, that there are small regions above the knot where fibre directions change sharply over very short distances. Data on RL4 are only available for small volumes (S4a–b), but it was found that data on RL3, which are available for larger volumes of wood (S3a–b), are sufficient for tracing tracheids with intermediate to large-sized lumen. Only small latewood lumen in transition wood and within the knot cannot be reliably traced in the data of RL3. This is illustrated and further discussed in the following section.

Traced fibre directions at the junction between branch and trunk

In this section, visualizations of traced fibres and patterns at the branch-stem junction are presented. The results are based on data from scanning of S3a and S3b on

RL3 (7 μm voxel size) and of S4b on RL4 (2.6 μm voxel size). Figure 8 presents the visualisation of fibres traced (Setting 5) within S3a and S3b along with a rendering of the data around the knot. The numbers 0–4 marked along a horizontal axis just below the knot in Fig. 8a indicate groups of fibres with different trajectories around the knot. The fibres at 0, represented by red curves within S3a, integrate from below the trunk with the lower part of the knot/branch. At 1, slightly to the right and now within S3b (in which a mix of colours is used to represent traced fibre directions), the fibre paths first curve around the knot before integrating with the branch at its lower right. Further to the right, at 2 and 3, fibres from below also bend/curve around the branch before they integrate with it further up on the right side. Between 3 and 4, the fibre paths follow a common curvature up until the region above the knot, i.e. the highlighted region of S3a, which is shown enlarged in Fig. 8c. Within this region, there is something like a ‘watershed’ for the flow of fibres such that the fibre paths below this integrate with the knot at its top, while the fibre paths above/outside this ‘watershed’ pass by the knot and continue up the trunk. In Fig. 8c, a black dashed curve is drawn to separate groups of fibres/curves above and below this ‘watershed’. The upper group of fibres in Fig. 8c represents fibres that pass by the knot on their way up the trunk of the tree, as indicated by black arrows. The lower group represents fibres that grow to integrate with the branch, as indicated by the green arrows. Note the very small curvature radius (only about 1 mm) by which fibres, on their path into the branch, change direction in the region above the knot. It can also be seen that curves representing fibres that integrate with the branch bend slightly upward before they continue into the knot at a somewhat lower vertical position. The few curves in the lower part of Fig. 8c that continue into the knot represent fibres that grew in an earlier year compared to those displayed (above the black dashed line) that do not integrate with the knot. Returning to the horizontal axis shown in Fig. 8a, it can be seen that fibre paths at position 4 and further to the right in S3b curve around the knot and continue up the trunk. Figure 8b shows, just like 8a, traced fibres within S3a and S3b, but from a different angle and without rendering of the knot, and the different paths described in Fig. 8a are again seen.

The images shown in Fig. 8d–e represent the same sub-volume as the one shown in 8c, but in different views and with more curves drawn in the images (Settings 3b and 3c were used for the tracing of the fibres represented in Fig. 8d and e, respectively). The perspective in Fig. 8d is looking at the tree from outside in the direction of the knot. Just as in 8c, black arrows are used to mark fibre paths that continue up the trunk and green arrows to indicate fibre paths on their way into the branch. White dashed curves are used to mark ‘watersheds’ (the more horizontal of the curves) and converging fibre paths (the more vertical of the curves). The density of drawn coloured curves (controlled by the settings parameter *Min.dist.*) is set to be low enough to enable a view *into* the volume in which individual curves can be seen and comprehended in 3D, yet high enough to indicate path directions everywhere within the volume. Note that, a vertical line, if drawn right through the centre of the knot, would *not* represent a line of symmetry. Rather, some fibre paths from the left and right side of the knot, respectively, converge at a position clearly to the left of the centre of the knot, as indicated by the two green arrows drawn in the lower part of the image. In Fig. 8e, a view from the side of the knot is shown. The annual rings

Fig. 8 Visualizations of traced fibres at the branch-stem junction. **a, b** Fibre directions traced within S3a and S3b in two different views. **c** Enlargement of volume above the knot, black and green arrows indicating fibre paths growing up the trunk and into the branch, respectively. **d, e** Fibre paths of the same sub-volume in a front view and a side view, respectively. White dashed curves mark 'watersheds' or conjunction of fibre paths that continue up the trunk and into the knot

are visualized by means of a rendering (the small image to the upper left in 8e shows the studied volume), reduced in size and free from fibre curves such that all annual rings can be seen, and it is evident that fibres are parallel with growth layer surfaces. As before, a black arrow is used to mark the fibre paths that continue up the trunk and a green arrow to mark the fibre paths that continue into the branch. The white dashed curve indicates the ‘watershed’, in this view, between the two main fibre paths. Note that, fibres following the different paths are still almost parallel to each other, in this view.

The fibre pattern in the surrounding of the knot, shown in Fig. 8, explains the strength of the connection between the branch and the tree. When the branch of the living tree is bent downward, for example by its own weight or the weight of snow, tensile stresses on the top side of the branch will be transferred by fibres in longitudinal fibre direction. The small zone in which the ‘watershed’ between fibre paths is located (best seen in Fig. 8d–e) is surrounded by parallel fibres, on each side of this zone (Fig. 8a–b) and also above the zone (Fig. 8c), that continue up the trunk and provide effective reinforcement such that fibres at the ‘watershed’ are not torn apart. Loading on the branch in any other direction, for example caused by wind, means that fibres at the branch junction on opposite side of the branch will be loaded in tension and compression, respectively, consistently in the longitudinal fibre direction, which is always the strongest direction of the wood material.

The traced fibres shown in Fig. 8 do not represent the fibres with smallest lumen grown in the latewood at the end of each growth season, but only medium size to large size fibres/lumen. This is because latewood fibres are too small to be traced in data on RL3. Thus, it is not yet certain that latewood tracheids follow the same fibre pattern as those shown in Fig. 8. In Fig. 9, however, data on both RL3 and RL4, from scanning of S3b and S4b (the latter cut out from the part of S3b closest to the knot), respectively, are visualised. Figure 9a shows only fibres with comparatively large lumen that would have grown in the beginning of each growth season; these were traced using Settings 5 and are represented by red curves. Comparing the traced fibres with the CT stack image on top of the considered volume showing annual rings indicates that, in between the layers of earlywood fibres, there are empty spaces within parts of the annual rings. Figure 9b shows fibres of intermediate-size lumen (traced in S3b with Setting 3a) and small-size lumen (traced in S4b with Setting 1), represented by yellow and blue curves, respectively. In the enlarged image of Fig. 9b, it can be clearly seen that fibres with different size lumen follow similar paths. This is also observed in Fig. 7 for a region just above the knot within S4a on RL4. Thus, the present investigation shows no indication of changing fibre paths over growth seasons in Norway spruce, as was suggested by Shigo (1985). Rather, the presented results support the findings of Nelly (1991).

Conclusion and further work

X-ray micro-CT imaging and data processing have been employed to investigate the transition zone where the branch connects with the trunk in Norway spruce. In particular, the size of fibres, local fibre directions and mapping of the fibre direction

Fig. 9 Visualization of fibres with different size of lumen traced within S3b and S4b. **a** Only fibres with large lumen traced (red curves), latewood areas are left empty. **b** Fibres with medium size and small lumen represented by yellow and blue curves, respectively. The latter traced on RL4 within S4b. Fibres grown at different times of the year follow the same growth pattern

patterns in clear wood and branch wood have enabled the 3D structure of the wood to be clarified in this complex region. A wood specimen ($75 \times 75 \times 107 \text{ mm}^3$) containing an entire knot from pith to bark was sawn from a selected tree. After drying, the specimen was scanned with micro-CT to provide 3D imaging data of the entire knot and surrounding clear wood with a voxel size of $52 \mu\text{m}$. Subsequently, smaller specimens were cut out from this initial, large specimen and scanned again with higher resolution, before even smaller specimens were cut out, and so on. Altogether, four levels, with successively smaller specimens and imaged with successively higher resolutions, were obtained. The smallest specimens were imaged with a voxel size of $2.6 \mu\text{m}$. The position of smaller specimens within the larger ones was determined such that all specimens of different size could be accurately placed in relation to the others. It was found, based on S4a of RL4, that the size of clear wood tracheids was about 1.2 mm long and that smaller dimensions of lumen ranged from 20 to $50 \mu\text{m}$ and from 15 to $35 \mu\text{m}$ in the radial and tangential directions, respectively. Branch wood tracheids were about 0.5 mm long and smaller dimension lumens ranged from 5 to $25 \mu\text{m}$.

A computer algorithm was used to trace local fibre directions in the different 3D images such that even at the second highest resolution level, RL3 ($7 \mu\text{m}$ voxel size), tracheids with large- to intermediate-size lumen, could be accurately traced for two specimens, S3a and S3b, each $9.5 \times 9.5 \times 50 \text{ mm}^3$ in size, covering almost the entire transition zone between knot and clear wood. With the highest resolution images, RL4 ($2.6 \mu\text{m}$ voxel size), tracheids with the smallest lumen, and even rays, were successfully traced for two specimens, S4a and S4b, each $3.5 \times 3.5 \times 20 \text{ mm}^3$ in size.

The data obtained were utilized to trace, visualize and understand local fibre orientation in the connection between branch and trunk. It was shown that fibre paths from regions below the knot integrate with the branch/knot at the bottom or on its left or right side, depending on the start position of the fibre path in lateral direction below the knot. Fibre paths starting slightly more to the left or right below the knot curve around the knot all the way up to the region just above the knot where they divide into two possible paths, one that continues into the branch on its top side and one that continues up along the trunk. It is in this region, just above the knot, that the most intriguing fibre patterns are found. Fibres in this region, following the path into the branch on its top side, curve, with a radius of about 1 mm or even smaller, in both vertical and horizontal directions just before the paths continue into the knot. Images of the traced fibres in different views allowed curvatures and ‘watersheds’ between fibre paths to be illustrated and it was shown that there is no abrupt change of fibre pattern between latewood and earlywood, rather there is a continuous change of fibre direction across annual layers. The fibre pattern explains the strength of the mechanical connection between trunk and branch in Norway spruce. Regardless of whether the branch is loaded vertically by self-weight and snow, or laterally by wind, stresses are directed in the strong longitudinal fibre direction.

Overall, the new detailed knowledge obtained from the 3D, multi-resolution imaging has enabled better explanation of the known, remarkable strength of the connection between branch and trunk in Norway spruce. In future work, the traced fibre directions could be expressed in mathematical functions and utilized in models to calculate and predict stiffness and strength both of branch-stem connections in living trees and of sections with knots in sawn timber.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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