An Improved Method for the Segmentation of Roots from X-ray Computed Tomography 3D Images: Routine v.2

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Methodology

Keywords: High-throughput root phenotyping, Image analysis, Root segmentation, Root system architecture, Cylindrical feature detection, X-ray computed tomography , Root diameter

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An improved method for the segmentation of roots from X-ray computed tomography 3D images: Routine v.2

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Abstract

Background

X-ray computed tomography is acknowledged as a powerful tool for the study of root system architecture of plants growing in soil. In this paper, we improved the original root segmentation algorithm “Routine” and present its succeeding version “Routine v.2”. In addition to grey value information, Routine algorithms are based on shape detection of cylindrical roots. Both algorithms are macros for the ImageJ software and are made freely available to the public. New features in Routine v.2 are (1) a pot wall detection and removal step to avoid segmentation artefacts for roots growing along the pot wall, (2) a calculation of the root average grey value based on a histogram analysis, (3) an automatic calculation of thresholds for hysteresis thresholding of the tubeness image to reduce the number of parameters and (4) a false negatives recovery based on shape

criteria to increase root recovery. We compare the segmentation results of Routine v.1 and Routine v.2 with the results of root washing and subsequent analysis with WinRhizo. We use a benchmark dataset of maize roots (*Zea mays* L. cv. B73) grown in repacked soil for two scenarios with differing soil heterogeneity and image quality.

Results

We demonstrate that Routine v.2 outperforms its preceding version in terms of root recovery and enables to match better the root diameter distribution data obtained with root washing. Despite a longer processing time, Routine v.2 comprises less user-defined parameters and shows an overall greater usability.

Conclusion

The proposed method facilitates higher root detection accuracy than its predecessor and has the potential for improving high-throughput root phenotyping procedures based on X-ray CT data analysis.

Keywords

High-throughput root phenotyping - Image analysis - Root segmentation - Root system architecture - Cylindrical feature detection - X-ray computed tomography – Root diameter

Abbreviations

Computed Tomography (CT); Root System Architecture (RSA); Magnetic Resonance Imaging (MRI); Non-Local Means (NLM); Region of Interest (ROI); Root Length Density (RLD); Absolute Difference Transform (ADT); WinRHIZO (WR);

39 **Introduction**

40 X-ray computed tomography (CT) is acknowledged as a powerful tool for the
41 study of root system architecture (RSA) of plants growing in soil. However, the study of
42 the root system architecture is only possible after performing root segmentation, i.e. the
43 binarisation of the greyscale data into root voxels and background voxels. Root
44 segmentation is often regarded as a tedious and difficult task as its success depends on
45 several factors such as the image resolution, the signal to noise ratio during image
46 acquisition and the grey value contrast between the roots and all other surrounding
47 features in soil [1].

48 In the past years, many methods have been developed to segment and visualise
49 roots in tomograms acquired with X-ray CT [1-16]. Some algorithms rely on simple
50 thresholding methods [8]. With these methods, the roots are segmented based on a
51 histogram analysis and a grey value criterion. These methods usually fail at segmenting
52 roots properly because of the overlapping grey value of roots, water, organic matter and
53 the soil matrix. The grey value at the edges of roots, water, organic matter and soil
54 matrix also show gradual changes of intensity spanning several voxels rather than a
55 crisp intensity step [17]. This effect, known as the “partial volume effect”, is also
56 responsible for poor segmentation results when using simple thresholding methods.
57 More advanced thresholding methods rely on the use of adaptive local thresholding
58 values (also referred to as “Region growing”) which use an additional connectivity
59 criterion to binarise the root and background voxels [3]. For both the simple and the
60 adaptive thresholding methods, there is an inherent trade-off to be made by the user. If

the grey value range assigned to roots bounded by two thresholds is too broad, oversegmentation may occur (i.e. segmented root edges extend into the surrounding features and appear frayed) and the false positives need to be removed through user-interaction which is a subjective, tedious and time-consuming task. Inversely, if this grey value range is too narrow, an important loss of roots may occur which biases the RSA analysis of the scanned sample. To tackle the issue of overlapping grey values of roots and other materials, root tracking methods such as the “RooTrak” algorithm have been developed [10]. With this method, the volumetric data is viewed as a sequence of x - y cross-sectional images aligned along the z axis. As the 3D stack is explored, root cross sections appear to move in the image and such “movements” can be used to reconstruct the root system. Methods relying on deep-learning algorithms and multi-scaled based approaches have also become common in X-ray CT and MRI imaging. Promising applications of deep-learning for the segmentation of roots from X-ray CT data were recently demonstrated by [13].

Gao et al. [6] proposed a new algorithm to segment root systems growing in soil by exploiting a typical morphological characteristic of the roots, i.e. their cylindrical shape. This approach was first introduced for vessel detection in medical imaging [18]. The vessel enhancement filter was later adopted to segment roots in 3D Magnetic Resonance Imaging (MRI) data [19]. The rationale of this method is that the cylindrical shape of roots is unique among all materials and features found in soil. The shape-based semi-automated algorithm is named “Rootine” [6] and has shown to outperform the “Root1” [4] and “Region growing” [3] methods in terms of root recovery and

segmentation accuracy. This demonstrated promising future applications of the algorithm for high-throughput root phenotyping based on X-ray CT data analysis. However, the Routine algorithm relies on a substantial number of parameters to be calibrated by the user. Moreover, Routine suffers from the fact that some of the parameters and their effects are difficult to identify and to interpret by a non-experienced user.

In this paper, we aim at developing an improved method for the segmentation of roots from 3D X-ray CT images that overcomes the aforementioned drawbacks of Routine. The objectives of this work are then to develop a new Routine version, i.e. “Routine v.2”, segmentation algorithm for which the segmentation accuracy and the user friendliness are increased. Specific objectives are to propose a second version in which:

- (1) the root recovery is higher;
- (2) the segmented root diameters are better captured;
- (3) segmentation artefacts are reduced;
- (4) the number of tunable parameters is reduced;
- (5) the parameters are related to root properties (i.e. their grey value, shape and connectivity).

The ability of the new segmentation algorithm to fulfill these criteria is evaluated by systematic comparison with the former algorithm Routine (which will be referred to as “Routine v.1”) and the results obtained by conventional, destructive root sampling and analysis of the washed-off roots with the software WinRHIZO. The comparison with

other state-of-the-art method is not carried out again, as the previous version of Rootine already demonstrated to outperform them [6]. In addition, a comparison between both algorithms is made by considering aspects such as the runtime and the overall usability of the algorithms. In that respect, the benchmark dataset of the “worse case” scenario presented in [6] and briefly described in the method section of this paper is used. This benchmark dataset was chosen to test Rootine v.2 as it presents several challenges to overcome, namely the presence of high soil heterogeneity, a poor quality of the images (i.e. a low number of projections during image acquisition) and a rather low image resolution as compared to the diameter of the roots to segment. The two first challenges contributed to a deterioration of the signal to noise ratio whereas the third exacerbates the partial volume effect at the edges of the fine roots. In the study of [6], these challenges led to a rather low root recovery of (i.e. 29 %) in comparison with conventional root sampling. These challenges combined make the benchmark dataset of [6] a perfect candidate for further testing and improvements of root segmentation algorithms. In order to show that the improvements in segmentation quality are not solely due to an overfitting of the Rootine v.2 for this particular dataset, we also demonstrate the performance of the new version on the so called “best case” scenario dataset of [6]. In this “best case” scenario, the soil and the scan settings were chosen in order to create low soil heterogeneity and a high signal to noise ratio. Those two aspects contributed to a robust estimation of root length (i.e. 99 % of recovery) in comparison with root washing and analysis with WinRHIZO.

Material and Methods

Plant growth conditions and destructive sampling

Maize plants (*Zea mays* L. cv. B73) were grown in repacked soil sieved down to ≤ 2 mm particle size. The plants were grown in a climate chamber for 21 days in cylindrical containers of 7 cm inner diameter and 23 cm height. Six plants were analyzed for each scenario. One day after X-ray CT scanning, the plants were harvested and the pots were cut in several layers of 4 cm. The roots in those layers were washed off with deionised water and stored in a 50% ethanol solution prior to analysis. In order to assess root length density (RLD) for each layer, root samples were scanned with a flatbed scanner (EPSON perfection V700) and the obtained images were analyzed with WinRHIZO Pro™ (Version 2019a, Regent Instruments, Canada). In total, twelve layers were investigated for each scenario (i.e. 2 per growing pot, one at the top and one at the bottom). For detailed information on the plant growth conditions and the destructive root sampling method, the reader is referred to [6].

Workflow of Rootline v.2

The workflow of Rootline v.2 is synoptically shown in Fig. 1 where the novelties of the algorithm are highlighted in blue and the steps and/or parameters that were modified from the original Rootline v.1 are shown in purple.

Image acquisition

3D X-ray CT images were acquired and reconstructed into an 8-bit greyscale 3D tomogram having a voxel size of 45 μm . The 8-bit conversion allows saving space

without considerable loss of information. During the 8-bit conversion, contrast was optimized using a percentile stretching method, i.e. 0.2 % of the darkest and brightest voxels are set to 0 and 255 respectively. A linear stretching is applied for all grey values between 0 and 255. Considering the geometry of the panel detector of the X-ray CT device (X-TEK XTH 225, Nikon Metrology), pots were scanned at two depth intervals (i.e. a bottom and top depth) making sure that an overlapping region was present. For more information regarding the image acquisition procedure, the reader is here again referred to [6].

Preprocessing

Before concatenation of the bottom and the top images, the overlapping regions in both scans are removed using the “Slice remover” function available in the free software ImageJ [20]. After concatenation, the obtained image comprises 3000 voxels in the z dimension, 1750 voxels in the x and y dimension and has a size $\approx$ 8.6 GB. The vertical extent of the stack is 13.5 cm. At the boundary of the two stitched images in the concatenated stack, a grey value discontinuity is present due to an illumination drift caused by the CT hardware (see Fig. S2 in [6]). This grey value discontinuity is corrected for using the “Attenuation correction” plugin [21] in ImageJ. This correction applies a linear transformation of grey values to each slice of the stack in order to make the average and standard deviation of the background constant (i.e. equal to that of a reference slice) throughout the stack [21]. Note that this grey value discontinuity is specific to the CT hardware used in the benchmark dataset of [6] and that this step might not be necessary with other set-ups.

Once the grey value discontinuity of the stitched stack is corrected for, a filtering step is performed with a “3D Non-local Means (NLM)” filter [22]. This filtering step is performed with a plugin available in the ITK library [23]. This filter was chosen as it is fast [22] and can easily be incorporated in the workflow thanks to its standalone application. Note that in contrast to [6] (see Fig. S3 in [6]), we converted the images to 16-bit and added a constant grey value offset of 50000 to avoid the change of contrast inherent to the use of this filter. The change in contrast is an outcome of the Rician noise model implemented in this filter [22] as it was originally implemented for MRI images. Avoiding the non-linear contrast enhancement for the low attenuation materials makes the results directly comparable to other softwares implementing a 3D NLM filter (e.g. Avizo™). The strength of the filtering is determined by the parameter “Contrast threshold” (t_{con}) which needs to be given by the user as an input parameter. It is adjusted to the standard deviation of the image noise assessed by the histogram analysis. Similarly as in [6], the remaining parameters of the 3D NLM filter were set to default. The result of the 3D NLM filtering can be assessed by comparing the original greyscale data (Fig. 2a) and the filtered image (Fig. 2b).

Rootine v.2 features a new pot wall detection step. This step serves two purposes. The first purpose is to create a mask which will exclude the pot wall from the data to segment and the second is to use the characteristic grey value of the pot wall to generate a peak in the histogram. The generated peak is used later on for the calculation of the average root grey value ($\bar{v}_r$) during a background removal step. In order to create a mask which excludes the pot wall, the coordinates of a circular region

191 of interest (ROI) bounded within the pot wall limit need to be defined manually at three z
192 slices of the stack, i.e. at the first, the middle and the last slices. Those three sets of
193 coordinates need to be given as input. The coordinates of the bounded ROI for all z
194 slices are then linearly interpolated from the given x-y coordinates of the bounded ROI
195 at the three z slices. This allows creating a 3D mask, i.e. a mask whose boundaries in
196 the x and y dimensions will move as the stack is explored in the z dimension. Creating a
197 3D mask is necessary to cope with pots being tilted during the X-ray CT scanning. Note
198 that, at the resolution used in the benchmark dataset and considering the pot height, a
199 certain tilt of the pots is always present. For tilted pots, a 2D mask would result either in
200 masking the roots growing along the pot wall or in the inclusion of the pot wall in the
201 data to segment. With the 3D mask, a logical “And” operation is used on the filtered
202 image in order to remove the pot wall from the data to segment. Once the bounded ROI
203 is calculated for all z slices, an extended ROI is created by simple extension of the
204 bounded ROI by 50 voxels (Fig. 3.a). The extended ROI serves the purpose of including
205 the pot wall in the histogram analysis of the stack so that a characteristic peak is
206 generated. After extracting the histogram of the extended ROI, a function searches for
207 the maxima in the lowest part (i.e. from 0 to 128) and in the highest part (i.e. from 128 to
208 255) of the histogram. This function then retrieves the grey value of those maxima, i.e.
209 P1 and P2 which correspond to the average grey value of the pot wall and of the soil
210 matrix respectively (Fig. 3.b). Those two values are used further down in the workflow
211 during the background removal step.

An edge enhancement step is then applied with the “Unsharp Mask” filter in ImageJ. “Unsharp Mask” filters enhance the local contrast between root edges, the surrounding soil matrix and pores [24]. The degree of edge enhancement is controlled by two input parameters. “Blur radius” is the standard deviation of the blur radius of the Gaussian filter kernel and “Mask weight” determines the strength of the filtering. The result of the edge enhancement step can be assessed by comparing the image filtered with 3D NLM (Fig. 2b) and the image after “Unsharp Mask” (Fig. 2c).

A new feature of the Routine v.2 algorithm is to apply a background removal step. During the background removal, every voxel whose grey value deviates too much from the average root grey value is masked out. This operation is performed in a three steps procedure. During the first step, a calculation of the root average grey value is carried out using the previously identified characteristic peaks of the pot wall and of the soil matrix. Assuming that a shift of P1 and P2 would result in similar shift of $\bar{v}_r$, $\bar{v}_r$ can be calculated for every image of the dataset using Eq. 1,

$$\bar{v}_r = f_r \cdot (P2 - P1) + P1 \quad \text{Eq. 1}$$

where f_r is the root grey value factor which has to be determined a priori on a representative test image. The first step allows coping with differently contrasted images in the dataset. Differences in image contrast are due to the percentile stretching method used during the 8-bit conversion when reconstructing the tomograms. Once $\bar{v}_r$ is calculated, a second step named “Absolute Difference Transform” (ADT) is applied. The rationale of this step is to brighten the grey value of the roots and darken all background

233 voxels including pores and soil matrix, as both materials have a grey value different
234 than $\bar{v}_r$. For every voxel, this is done by computing Eq. 2,

$$235 \quad v_{ADT} = 255 - |v_f - \bar{v}_r| \quad \text{Eq. 2}$$

236 where v_{ADT} is the voxel grey value after the ADT and v_f is the voxel grey value prior
237 ADT. The background is then masked out by thresholding the image with a threshold
238 value (t_{ADT}) calculated with Eq. 3,

$$239 \quad t_{ADT} = 255 - \frac{R_r}{2} \quad \text{Eq. 3}$$

240 where R_r is the root grey value range centered around $\bar{v}_r$. R_r has to be determined a
241 priori on a representative test image and has to be given as a input parameter. The
242 image obtained after background removal serves as the input image for the subsequent
243 root segmentation step. This three-step procedure replaces the simple pore masking
244 step in Routine v.1. The result of the background removal step can be assessed by
245 comparing the image after edge enhancement (Fig. 2c) and the image after background
246 removal (Fig. 2d).

247 *Root segmentation*

248 A specificity of the Routine algorithms is to segment roots by exploiting one of
249 their inherent characteristics, i.e. their cylindrical shape. To do so, the “Tubeness filter”
250 available in ImageJ is used. In brief, the tubeness filter performs a smoothing of the
251 image and produces an image in which the grey values are directly related to how
252 similar an object is to a cylinder. Generally, a scaled approach is adopted, i.e. the same
253 image is filtered with Gaussian filters of different strength determined by their σ values,

segmented and then combined. For low σ values, roots of small diameters will evoke high grey values after tubeness filtering whereas bigger roots will either appear hollow or display low grey values after tubeness filtering. Increasing the σ values of the tubeness filter then results in the opposite effect, i.e. roots of greater diameter appear brighter whereas roots of smaller diameter will vanish. The obtained series of images are then thresholded using the “3D Hysteresis thresholding” method available in the 3D ImageJ Suite [25] and then combined to reconstitute the full root system. Hysteresis thresholding is a segmentation method requiring two thresholds. With an upper threshold, seed regions definitively belonging to roots are determined. The upper threshold (t_{hys}^{high}) is less relevant for segmentation accuracy and can be set a priori. From the upper threshold, a region growing process connects all voxels brighter than a lower threshold (t_{hys}^{low}). This region growing process improves edge continuity in gradient images [26] and the class assignment of partial volume voxels [27], thereby reducing the presence of false positives.

In this work, we introduce a new method to estimate the lower threshold applied during hysteresis thresholding. This estimation is based on the measurement of root diameters present in the image to segment. The link between the lower threshold applied during hysteresis thresholding and the root diameters has been made by analyzing carefully the results of the tubeness filter for increasing σ values applied on the same image of a hypothetical root (Fig. 4a). This was achieved with the following sequence of operations. First, a root of a diameter d_r is created by drawing a white cylinder on a black background. Then, this root image is filtered with tubeness of

increasing σ values. Note that the absolute value of the tubeness intensity depends on the gradient magnitude and hence the level of smoothing. Therefore, the contrast in the tubeness filter results were normalized, i.e. the highest grey value after filtering is set to 255 during conversion from 32-bit to 8-bit. In order to generalize the obtained results, we introduce the normalized smoothing strength q which is equal to

$$q = \frac{\sigma}{d_r} \quad \text{Eq. 4}$$

where σ is the smoothing strength of the tubeness filter and d_r is the root diameter. Both parameters are expressed in number of voxels. For each q value, a grey value transect along the root diameter axis is plotted (Fig. 4b). It is shown that, for low q values, the filtered root appears hollow and has symmetrical grey value peaks on both sides of the root diametrical axis. For q values greater than 0.125, the grey value transects have a concave parabolic shape with their symmetrical axis centered on the root diametrical axis. For a given q value, the grey value at the intersection of the parabola and the original root outline (i.e. the vertical blue dashed lines in Fig. 4a and 4b) corresponds to the optimal lower threshold (t_{hys}^{opt}) to use during hysteresis thresholding in order to precisely capture the original root diameter. To formalize the calculation of t_{hys}^{opt} , we retrieved t_{hys}^{opt} (i.e. the colored dots in Fig. 4b) for all q values and fitted a regression model (Fig. 4c) which describes best the relationship between those two parameters (i.e. highest possible R^2 values). We then calculated t_{hys}^{opt} for $q = 0.5$ using the model regression (i.e. $t_{hys}^{opt} = 79$, see the dashed line in Fig. 4c). With the optimal lower threshold calculated and with a measurement of the diameter of the root to segment,

the sigma value of the tubeness filter can be estimated (Eq. 5). It is important to note that, in case of image rescaling, the resolution factor (f_s) needs to be accounted for in the determination of d_r . Thus, Eq. 4 is recast to:

$$\sigma_i = q \cdot d_{r,i} \cdot f_s \quad \text{Eq. 5}$$

where σ_i is the smoothing strength of the tubeness filter to use to properly segment a root of a certain diameter $d_{r,i}$, q is the normalized smoothing strength and f_s is the resolution factor. For the segmentation of fine roots, we segmented the images at the original resolution (i.e. $f_s = 1$) whereas the coarse roots were segmented with a downscaled input image (i.e. $f_s = 0.5$) in order to reduce processing time without considerable loss of information. To determine values for $d_{r,i}$ a priori, we adopted an incremented approach to account for the continuous distribution of root diameters (Fig. 5). This approach requires three parameters, namely the “Minimum root diameter”, the “Root diameter increment” and the “Maximum root diameter”. All three parameters are expressed in units of number of voxels. The minimum ($d_{r,\min}$) and maximum root diameter ($d_{r,\max}$) were determined by measuring the diameter of the finest and biggest root in the image with the “Measure” tool available in ImageJ. The root diameter increment ($d_{r,\text{inc}}$) parameter refers to the increment at which roots of increasing diameters are detected. Here, we set a root diameter increment value equal to four voxels, which yields an incremented σ value of one at the coarse resolution according to Eq. 5. With these three parameters, an incremented calculation estimates the appropriate sigma values of tubeness for each resolution and scale considered and root diameters targeted. After filtering with tubeness and subsequent segmentation with

hysteresis thresholding, the results were combined into one image through a logical “Max” operation, i.e. a voxel is assigned to the root class if it is assigned to roots in at least one resolution or scale. This updated approach replaces the fixed scales and manually defined t_{hys}^{low} for each scale in Routine v1. The result of the root segmentation step is in Fig. 2e.

Postprocessing

The postprocessing steps aim at removing artefacts which occurred in the course of segmentation. Such artefacts may include for instance segmented particulate organic matter or isolated pores whose grey values are in the same range as the one of the roots. First, a 3D Median filter is applied on the segmented images in order to smoothen the root surfaces. The degree of filtering is determined by the kernel size of the filter which needs to be given as an input parameter. On one hand, this filtering operation is favorable as it trims some oversegmentation voxels extending from the roots into the surroundings. On the other hand, this trimming also causes some fine root segments to be disconnected from the root system. After 3D Median filtering, the unconnected objects are removed using a connectivity criterion. This operation is performed with the “Keep Largest” function available in the “MorpholibJ” plugin library [28]. Prior to “Keep Largest”, an extra slice is added at the top of the stack to ensure the connectivity of all root segments from top to bottom. This is necessary when the seed from which all roots emerge is not part of the image. In the case of *Zea mays*, adding this step is essential as it allows keeping the brace and crown roots which do not directly emerge from the seed but always enter the ROI from the top.

A new feature of Routine v.2 is to implement a “false negatives” recovery step. This step labels and evaluates every object unconnected to the root system and test whether it fulfills shape criteria which evoke typical shape of roots. Those unconnected objects are either segmented clusters of pores and/or segmented particulate organic matter (i.e. false positives) or root segments which were disconnected due to the trimming effect of the previously applied 3D Median filter (i.e. false negatives). Here, we evaluate every unconnected object based on two criteria, i.e. its “Vesselness” and its size. To evaluate the vesselness, we adopt a simplified formulation of the vesselness function proposed by [18] and derive a “vesselness score” of individual objects. This is based on the analysis of the length of the semi-axes of fitting ellipsoids to binary objects instead evaluating the Hessian matrix (i.e. the 2nd derivative of grey values) of each voxel. The semi-axes of the fitting ellipsoids are noted λ_1 , λ_2 and λ_3 . By convention and in order to make abstraction of the local orientation of the considered object in the 3D space, we pose $\lambda_1 \leq \lambda_2 \leq \lambda_3$. For every object, we then compute the following geometrical ratios:

$$Rb = \frac{\lambda_1}{\sqrt{\lambda_2 * \lambda_3}} \quad \text{Eq. 6}$$

$$Ra = \frac{\lambda_2}{\lambda_3} \quad \text{Eq. 7}$$

The first ratio accounts for the deviation from a blob-like structure. For a blob-like object (i.e. $\lambda_1 \approx \lambda_2 \approx \lambda_3$), Rb will attain high values whereas it will have low values for elongated objects (i.e. $\lambda_1 \approx \lambda_2 \ll \lambda_3$). The second ratio is essential for distinguishing between plate-like and cylinder-like structures. For a plate-like object (i.e. $\lambda_1 \ll \lambda_2 \approx$

λ_3), Ra will reach its maximum whereas it will be low for elongated objects. Based on the defined ratios, we evaluate how similar an object is to a cylinder by deriving the vesselness score (v) according to Eq. 8.

$$v = \exp(-Rb^2) \cdot \exp(-Ra^2) \quad \text{Eq. 8}$$

The vesselness score can have values ranging from 0.13 for a perfect sphere to ≈ 1 for an infinitely long cylinder. The relationship between the length of the semi-axes of the fitting ellipsoids, the calculated geometrical ratios and some properties of the vesselness score are illustrated in Fig. 6 for simple geometrical objects, i.e. a sphere, a plate and a cylinder. In addition to the vesselness criterion, a size criterion is used in order to exclude small objects which originate mostly from the noise level in the image and may by chance fulfill the vesselness criterion. The size criterion is given by a single value being equal to the greatest length of the semi-axes of the fitting ellipsoid, i.e. λ_3 . In practice, evaluating the unconnected objects is performed in three steps. All steps rely on operations available in the “MorpholibJ” plugin library. First, a label is assigned to every unconnected object via the “Connected Components Labeling” function. Second, for every unconnected object, the length of the semi-axes λ_1 , λ_2 and λ_3 of the fitting ellipsoids are computed with the “Analyze Regions 3D” function. Third, the vesselness score is calculated and assigned to each label using the “Assign Measure to Label” function. An object is then considered false negative only if the following conditions are met:

$$v_i > t_v \quad \text{and} \quad \lambda_{3,i} > t_s$$

where ν_i and $\lambda_{3,i}$ are the vesselness score and size score of the object i and t_v and t_s are the vesselness and the size threshold respectively. The vesselness and the size threshold are input parameters which need to be given and calibrated by the user. After discriminating between false positives and false negatives, the false negatives are added to the connected root system whereas the false positives are discarded. Fig. 7 illustrates this new approach of the postprocessing scheme implemented in Routine v.2. The effect of the post processing steps is shown in the difference between Fig. 2e and Fig. 2f.

Quantification and analysis

Following postprocessing, the images can be analyzed and quantified in terms of root length and diameter. The quantification of root length from X-ray CT data has to be preceded by a step of skeletonization which conducts a medial axis transform of the segmented root image. This results in an image where all roots are reduced to a 1 pixel wide object which makes the calculations of root length more reliable and faster. This is achieved by sequentially applying the “Skeletonize (2D/3D)” and “Analyze Skeleton (2D/3D)” methods available in the BoneJ plugin library [29] implemented in ImageJ. The root recovery is assessed by plotting the root length calculated after skeletonization of the segmented root system and the root length analyzed with WinRHIZO (WR). By imposing a line of best fit to the relationship between both root lengths (CT and WR), the root recovery and the error consistency (i.e. the slope and the coefficient of determination of the line of best fit respectively) can be evaluated. The quantification of the root diameter distribution is performed with the “Local Thickness” plugin available in

405 BoneJ. This method assigns to every root voxel a value corresponding to the diameter
406 of the largest sphere which locally fits into the root. The results of the local thickness
407 images are intersected with the skeleton images with a logical “And” operation. The
408 resulting images are skeletonized root systems where each medial axis voxel contains
409 the local root diameter information. This intersection is performed in order to avoid that
410 big roots contribute to more voxels than smaller roots in the histogram. The histogram of
411 the obtained images is then computed to retrieve the root length corresponding to every
412 diameter class. Note that, even though roots were destructively sampled at two different
413 layers for six replicates, the results are shown here by pooling all replicates and all
414 layers together for each scenario. For both the root length and the root diameter
415 distribution, the root length is normalized by dividing by the volume of the soil layer and
416 the results are expressed in terms of RLD.

417 *Summary of the workflow and its parameters*

418 This section concludes the description of the workflow. Fig. 1 and Table 1
419 provide an overview of the steps of workflow and the tunable parameters involved to
420 obtain a segmented root system from an input grey scale data acquired with X-ray CT.
421 Table 1 also list the effect and the sensitivity of the parameters on the segmentation
422 accuracy.

Results

Root recovery and root diameter evaluation

Rootine v.2 outperformed its preceding version by an increase of the root recovery up to 73 % of the total root length, against 29% for Rootine v.1 in the worse case scenario (Fig. 8a). The coefficient of determination is roughly equal for both algorithms (i.e. $R^2 = 0.76$ and 0.79 for Rootine v.1 and v.2 respectively). A 2D Maximum Z-Projection of a selected sample (circled in black in Fig. 8a) shows that the oversegmentation is low (Fig. 8b). Detected roots appear relatively smooth and there are barely any root voxels extending into their surroundings. In the 2D Maximum Z-Projection, some root segments are disconnected from the root system. Those segments are the ones added by the false negatives recovery step during postprocessing. Fig. 8b also shows that the gain in root length with Rootine v.2 is mainly contributed by additional fine roots (operationally defined as roots having a diameter $\leq 180 \mu\text{m}$). The increased fine root recovery is also reflected in the root diameter distribution (Fig. 9a). On top of a higher root recovery of the fine roots, Rootine v.2 also enabled to better capture the root diameter of the big roots (operationally defined as roots having a diameter $\geq 900 \mu\text{m}$) as compared to its preceding version. This can be seen on Fig. 9a where Rootine v.2 agrees better with WR data for the diameter classes larger than $900 \mu\text{m}$ as compared to Rootine v.1. The second peak corresponding to the primary roots only underestimates WR values by 4 voxels for Rootine v.2 whereas this second peak is completely absent for Rootine v.1. The better agreement of the root diameters captured by Rootine v.2 can also be assessed visually by superimposing the

segmented images of both algorithms and by directly comparing them with the greyscale X-ray CT data (Fig. 9b).

The root recovery in the best case scenario amounted to 114 % of the total root length against 99 % for Routine v.2 and Routine v.1 respectively (Fig. 8c). Again, the coefficient of determination is roughly equal for both versions (i.e. $R^2 = 0.92$ and 0.90 for Routine v.1 and v.2 respectively). Similarly as for the worse case scenario, a 2D Maximum Z-Projection of the segmented roots of a selected sample (Fig. 8b) offers a visual comparison of the segmentation results for both versions. Here again, the oversegmentation is low as roots appear smooth and devoid of any oversegmented voxels at their boundaries. The increase in root recovery is also mostly contributed by the addition of fine roots. The agreement of the root diameter distribution is equally good for both versions (Fig. 9c).

Discussions

Segmentation accuracy

In the worse case scenario, most of the roots that were missed by Routine v.1 and v.2 belonged to the category of the fine roots. This was expected considering that one of the main challenges of the benchmark dataset is a low image resolution as compared to the small diameter of the fine maize roots. Indeed, the analysis of the cumulative frequency of root diameter of the WR data shows that the fine roots comprise roughly 45 % of the total root length. Fine roots pose a tremendous challenge due to the presence of partial volume voxels at the boundary of the roots and their

surroundings. This challenge is even bigger when the contrast between the roots and their surroundings is low, which was true for the worse case scenario. We attempted to capture more fine roots at the original resolution by reducing the minimum root diameter to two voxels (i.e. resulting in a smoothing strength of $\sigma = 1$). However, this resulted in too much oversegmentation. Still, Routine v.2 was able to capture twice as much of the fine roots as compared to Routine v.1 in the worse case scenario. This can be attributed to the background removal step during preprocessing and to the false negatives recovery step performed during postprocessing. The background removal prevented the presence of false positives even when segmenting the images at the original resolution. In contrast, the soil heterogeneity and the low signal to noise ratio of the worse case scenario forced [6] to downscale the images by a factor of 2 prior root segmentation. It was argued by the authors that segmenting the images at the original resolution resulted in too much oversegmentation. By applying an adequate background removal operation, Routine v.2 was able to avoid oversegmentation while improving the recovery of fine roots considerably. On top of an adequate background removal, the ADT step increases the grey value intensities of the roots prior tubeness filtering which is favorable for their subsequent detection. The false negatives recovery step also contributed to a fair amount of the root recovery. By computing the root length density before and after this step, we evaluated this contribution. On average, it amounted to 25.5 % (± 1.9 % standard error, $n=12$) of the total RLD. So it explained more than half of the gain in root recovery between Routine v.1 (29%) and v.2 (73%).

In the best case scenario, the RLD inferred from X-ray CT was higher than the one measured with WinRHIZO. This could be an indication of the presence of false positives and/or oversegmentation at the boundaries of root voxels. We rule out the latter since the visual inspection of the images showed virtually no root voxels extending into their surroundings. The overestimation of root length with Rootline v.2 could be due to the false negatives recovery step during which some actual false “positives” were considered as being false “negatives” and added back to the root system. For the best case scenario, the contribution of this step to the total RLD was lower than for the worse case scenario and amounted to 12.5 % ($\pm$ 0.8 % standard error, n=12). The overestimation of the root recovery could also be due to the uncertainties associated with the root washing procedure and further analysis with WR. The Z-Projection shown in Fig. 8d is one of the samples whose X-ray CT RLD data point exceeded the RLD data measured with WinRHIZO (i.e. the sample circled in black above the 1:1 line in Fig. 8c). When taking a closer look at Fig. 8d, it is obvious that the increase in root recovery is due to the addition of real roots. During root washing, a soil sample is placed on a sieve, the soil is then washed off with water and the roots remaining on the sieve (here having a 1 mm mesh size) are picked with a tweezer and stored in ethanol prior to analysis. Some fine roots can easily go unnoticed on the sieve due to their size. The fact that the overestimation of WR data by Rootline v.2 occurs specifically for the fine roots is an indication supporting this argument. On top of the root washing, errors in WR data might be induced by an uneven distribution of the roots on the tray used for scanning. An uneven distribution of the roots might cause two fine roots located very close to each other to be detected as one root with a larger diameter instead. During the

analysis with the WinRHIZO software, a noise threshold value has to be set to exclude small dirt particles from the root length calculation. A high noise threshold value leads to smooth root surfaces but also results in the loss of fine roots. An improper setting of this parameter may then also induce errors. We rule out the effect of storing roots in ethanol on the WR results as this procedure has proved to be valid to conserve root samples without considerable influence on the measurements of root length (i.e. < 1% of underestimation) and diameter (i.e. 5 % of underestimation) [30]. Both the potential loss of roots during washing and the underestimated capture of fine roots by WinRHIZO could explain the overestimation of the root recovery in the best case scenario. Note that if the RLD data characterized with WR is underestimated, it is likely that the root recovery in the worse case scenario is overestimated.

The segmentation accuracy was evaluated based on quantitative aspects such as the root recovery and the comparison of root diameter distribution. Additionally, segmentation accuracy was also evaluated visually based on qualitative aspects, i.e. how accurately the root diameter outlines were segmented. With Routine v.1, the primary roots often showed irregular shapes. With Routine v.2, primary roots were segmented with a higher accuracy and showed a prominent circular shape when viewed in a 2D x-y cross section. This can be seen in Fig. 9b and 9d. This increase in accuracy can most likely be ascribed to the fact that more scales were considered during the tubeness filtering at the coarse resolution with Routine v.2. However, this difference in capturing the root diameter outlines was not big enough to be reflected in the root diameter distribution in the best case scenario. By comparing the results of Routine v.1

and v.2 visually, it was also noticeable that the latest version showed less false positives and less segmentation artefacts. Such segmentation artefacts include for instance the oversegmentation of roots growing along the plastic wall of the pot. Routine v.1 did not feature a pot wall detection and removal step. Since the root average grey value is close to the one of the pot wall (see the corresponding grey values of $\bar{v}_r$ and P1 in Fig. 3b), it was often observed on the results of Routine v.1 that segmented root were extending into the plastic wall. This was particularly true in the best case scenario.

Numbers of tunable parameters

One of the objectives of this work was to develop an algorithm in which the numbers of tunable parameters is reduced. Note that we consider as “tunable” the parameters which require adjustments and calibration when applied to other datasets (i.e. experiments with different plants, scan settings and/or soil heterogeneity).

In Routine v.1, we identified twelve tunable parameters in total, namely five for the preprocessing, six for the root segmentation and one for the postprocessing. For the preprocessing, one parameter was used for filtering the original greyscale image (i.e. the contrast threshold of the 3D NLM filter), two for edge enhancement (i.e. the blur radius and the mask weight of the Unsharp mask filter) and two for masking the pores by single thresholding (i.e. pores were masked with different thresholds at the original and at the coarse resolution). For the root segmentation step at the original resolution, one σ value and the corresponding t_{hys}^{low} for hysteresis thresholding method were used. For the root segmentation step at the coarse resolution, three σ values were used. The obtained results of the tubeness filtering were then merged and one t_{hys}^{low} for the

554 hysteresis thresholding method was used to segment the results of the coarse root
555 detection (this amounts to four parameters). The upper threshold for hysteresis
556 thresholding (t_{hys}^{high}) was kept constant and high enough for every tubeness filtering
557 scale and is thus considered non-tunable. For the postprocessing, Routine v.1 relied on
558 one parameter, i.e. the kernel size of the 3D Median filter.

559 In Routine v.2, the pore masking thresholds used by [6] were replaced by the
560 parameters the root grey value faction (f_r) and the root grey value range (R_r). The
561 parameters of the 3D NLM filter and the Unsharp mask filtering are used in the new
562 version. With the introduction of the automatic calculation of the σ values and t_{hys}^{opt} and
563 keeping t_{hys}^{high} high enough and constant for every tubeness filtering scale, the number
564 of parameters required for the root segmentation was reduced to two (i.e. $d_{r,min}$, $d_{r,max}$).
565 As they have been set once for the new root segmentation approach, we consider the
566 parameters q , f_s and $d_{r,inc}$ as “quasi-fixed” and therefore non-tunable. For the
567 postprocessing, a smoothening step of the root outline was performed with the “3D
568 Median” filtering step requiring one parameter (i.e. the kernel size). The false negatives
569 recovery step added two parameters, i.e. the vesselness (t_v) and the size (t_s)
570 thresholds. In total, the number of tunable parameters in Routine v.2 was reduced to
571 ten, i.e. five for the preprocessing of the image, two for the root segmentation and three
572 for the postprocessing. It is worth noting that more parameters are required to use the
573 full functionalities of Routine v.2. Such parameters include for instance the coordinates
574 of the ROI mask. Those coordinates need to be directly evaluated on the image and are
575 not considered to influence the segmentation results if appropriate values are given.

Runtime and overall usability of Routine v.1 and v.2

Besides the segmentation accuracy and the number of parameters, the assessment of the performance of a segmentation algorithm also has to take into account the time it takes to process the images. This is important for application in high-throughput root phenotyping based on X-ray CT data analysis. To process a stack having a dimension of 1750x1750x3000 voxels (in x, y and z dimension, size $\approx$ 8.6 GB), it took Routine v.1 3.8 hours to complete the preprocessing, the segmentation and the postprocessing steps. In comparison, Routine v.2 took 6.8 hours to complete the same steps. The second version is 1.8 times slower than the first version for the analyzed image size. This is mainly due to the consideration of more scales during tubeness filtering at the coarse resolution. For both algorithms, the evaluation of the runtime was performed on a workstation having 64 Intel® Xeon® Gold 6142 cores running at 2.60 GHz each. To this date, filtering with the tubeness filter represents the bottleneck of the workflow. This is related to the fact that the tubeness filter is only implemented in a single threaded fashion in the ImageJ software. There should be no fundamental constraint that would restrict its parallelisation and Routine (regardless of the versions) would benefit a lot from it. Note that there exists a multithreaded implementation of the tubeness plugin which was developed in the context of ImageJ Ops [31]. We have however not tested it. It is worth noting that both algorithms can be run in a user interaction-free mode (i.e. from the command line) once the parameters are adjusted. This provides an advantage and, in our opinion, reduces the necessity of having a fast algorithm as the macro can run in the background and/or overnight. When it comes to RSA studies, a longer runtime can be well accepted as long as the root recovery is

substantially increased. Despite the longer time required to segment images, we assume that the increase of the overall usability of Rootine v.2 can save the user some time for the adjustments of the parameters. Indeed, Rootine v.2 features input parameters which are linked to root physiological properties such as their grey value and their diameter. These tunable parameters are easy to adjust as they can be directly assessed visually on a test image.

Conclusion

Rootine v.2 has been developed for improved root segmentation accuracy in X-ray CT data. It exploits intrinsic properties of root systems such as the connectivity of root branches and the cylindrical shape of roots to distinguish roots from the background. It was demonstrated that Rootine v.2 outperforms its precursor version in terms of root recovery, which was already proved to be superior to other state-of-the-art root segmentation methods. The gain in root recovery could be mainly ascribed to the absolute difference transform of the greyscale data prior to shape detection with a series of tubeness filters and to a false negatives recovery step. The other major advancements of Rootine v.2 are (1) a new pot wall detection and removal step, (2) a calculation of the root average grey value based on a histogram analysis and (3) an automatic calculation of thresholds for hysteresis thresholding of the tubeness image. Moreover, the analysis of the root diameter distribution is readily integrated in the new version. The total number of tunable parameters for the entire workflow was reduced from twelve to ten. Rootine v.2, in comparison to Rootine v.1, functions less in a “black box” fashion as its parameters can be more easily interpreted and are easier to adjust.

621 The proposed method has the potential of improving high-throughput root phenotyping
622 procedures based on X-ray CT data analysis. Similarly as its preceding version, Routine
623 v.2 is a macro for the image processing software ImageJ and is made freely available to
624 the public.

625 **Declarations**

626 ***Ethics approval and consent to participate***

627 Not applicable

628 ***Consent for publication***

629 Not applicable

630 ***Authors' contributions***

631 MP and SS developed the Routine v.2 code. MP processed and analysed the X-ray CT
632 data. MP wrote the manuscript and produced the figures with the assistance of SS. EL
633 processed the WinRHIZO data. DV facilitated the research and provided assistance
634 with the writing and edition of the manuscript. All authors read and approved the final
635 manuscript.

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644 ***Competing interests***

645 The authors declare that they have no competing interests.

646 ***Data availability***

647 The Routine v.2 workflow is available as supporting information. The CT images (top
648 and bottom, approx. 4GB each) of one pot of the worse case scenario are provided
649 through the file link: <https://nc.ufz.de/s/MyPPgjFJZe6nNxK> (password: Routinev2). for
650 testing reproducibility. The temporary file link will be replaced by a permanent archive
651 link after manuscript acceptance. The entire datasets used during the current study are
652 available from the corresponding author on reasonable request.

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656 **Tables**

657 **Table 1 Summary of the parameters, their values, their effects and their sensitivity**
658 **on the segmentation accuracy.**

Step	Parameter	Value		Effect	Sensitivity on the segmentation accuracy
		Worse case	Best case		
Image filtering	Contrast threshold (t_{con})	60	60	Controls the degree of smoothening (i.e. noise removal) of the input image.	Medium
Edge enhancement	Blur radius	1	0.9	Both parameters control the degree of sharpening of the image, i.e. increase the contrast at the boundaries between roots and pores and soil matrix.	High
	Mask weight	0.7	0.8		
Background removal	Root grey value factor (f_r)	0.10	0.18	Sets the grey value of the roots.	Very high
	Root grey value range (R_r)	65	70	Controls the root grey value window around the root grey value. If set too high, overestimation of roots into their surroundings will occur. If set too low, loss of roots will occur.	Very high
Detect fine roots	Minimum root diameter ($d_{r,min}$)	4	4	Controls the root recovery of the fine roots. If set too high, the fine roots are not detected. If set too low, oversegmentation may occur depending on the noise level of the image.	High
Detect coarse roots	Maximum root diameter ($d_{r,max}$)	28	28	Controls the accuracy of the root diameter outline of the biggest root. If set too high, the diameter of the biggest root is overestimated and computational time is wasted. If set too low, the diameter of the biggest root	Medium

				is underestimated.	
False positives removal	Kernel size of median filter	3	2	Controls the degree of smoothing of the roots and trimming of over segmented voxels. If set too high, root loss occurs whereas low values result in the presence of many false positives.	High
False negatives recovery	Size threshold (t_s)	25	25	Both parameters control the quality of the false negatives added in the root system. If set too low, many false positives are considered negatives, adding noise to the results. If set too high, root loss occurs.	High
	Vesselness threshold (t_v)	0.85	0.9		High

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Figure legends

Fig. 1 Synoptic view of the Routine v.2 workflow including the comparison with Routine v.1.

Fig. 2 Results of the steps of Routine v.2 for a subvolume of the worse case scenario; (a) The original greyscale image; (b) The obtained image after denoising with the 3D NLM filter; (c) The obtained image after performing edge enhancement of subfigure b; (d) Resulting image after background removal via an absolute difference transform on subfigure c; (e) Results of the root segmentation applied on subfigure d before applying postprocessing steps; (f) Segmented roots after applying the postprocessing steps on subfigure e.

Fig. 3 Mask creation and calculation of the average root grey value based on characteristic peaks. Subfigure (a) depicts the drawing of a circular ROI bounded within the pot wall (red circle) on a 2D section of the worse case scenario. The bounded ROI serves the purpose of creating a mask. By extension of this bounded ROI by 50 pixels, an extended ROI is created (blue line); (b) Histogram of the bounded and extended ROI illustrated in subfigure a. The extended ROI serves the purpose of creating a peak in the histogram which will be used to calculate the average root grey value.

Fig. 4 Estimation of σ values of the tubeness filters and the optimal lower thresholds of hysteresis thresholding. (a) Results of the tubeness filter on a hypothetical root of a diameter d_r obtained for different q values. The dashed blue lines show the original root outline whereas the solid yellow lines show the position of the transects used to plot the grey values along the root diameter axis; (b) Transects of the grey values along the root diameter axis for some of the q values shown in subfigure a. The color dots at the intersection between the root outline and the grey value parabola

781 correspond to t_{hys}^{opt} for a given q value; **(c)** Line of best fit imposed on the couple of
782 points q and t_{hys}^{opt} . In this study, we calculated t_{hys}^{opt} corresponding to $q = 0.5$ using the
783 model regression. The calculated value is indicated by the dashed line (i.e. $t_{hys}^{opt} = 79$).

784 **Fig. 5 Approach for the detection of roots of increasing diameters at the fine and**
785 **coarse resolutions.**

786 **Fig. 6 Vesselness score, Rb and Ra values for a sphere, a plate and a cylinder.**

787 **Fig. 7 Illustration of the postprocessing steps implemented in Routine v.2.** First, a
788 3D Median filter is applied on the results of the root segmentation step. Then, all
789 connected objects are kept by applying the “Keep Largest” function. In order to ensure
790 full connectivity of the roots at the top of the stack, a slice is added at the top (left-hand
791 side of the figure). The remaining unconnected objects are subject to a test evaluating
792 their shape, i.e. their “vesselness” and size. This is illustrated here by showing a Z-
793 Projection of a 400x400x400 image from the best case scenario dataset (right-hand
794 side of the figure). The green scale bar indicates the vesselness score whereas the red
795 scale bar indicates the size score. The intensity of the yellow color depicts the
796 combination of these two scores. If an object meets both the vesselness and size
797 threshold, it is considered as a false negative and will subsequently be added to the
798 connected root system. If not, it will be considered as a false positive and will thereafter
799 be discarded.

800 **Fig. 8 Root recovery of Routine v.2 for the worse and best case scenario. (a&c)**
801 Comparison with the former Routine v.1 and the root length determined with destructive

sampling and scanning of washed-off roots (WinRHIZO) for the worse case and the best case scenario respectively. The dashed line indicates the 1:1 line. **(b&d)** Visual comparison of the segmented root systems obtained with Rootine v.1 and Rootine v.2 for the corresponding sample circled in black on subfigures a and c for the worse case and the best case scenario respectively. Roots detected by both algorithms are depicted in black, the ones only detected by Rootine v.2 are shown in blue, whereas roots only detected by Rootine v.1 are shown in red.

Fig. 9 Root diameter distribution and root outline accuracy for the worse and best case scenario. (a&c) Root length density distribution as a function of root diameter for Rootine v.1 and v.2 and the destructive sampling data obtained by scanning washed-off roots (WinRHIZO) for the worse case and the best case scenario respectively. The semitransparent ribbon denotes the standard error of the measurements (n=12). **(b&d)** Visual comparison of the segmented root diameter outlines for both Rootine v.1 and Rootine v.2 supported by the original X-ray CT greyscale data for the worse case and the best case scenario respectively. Roots detected by both algorithms are depicted in black, the ones only detected by Rootine v.2 are shown in blue, whereas roots only detected by Rootine v.1 are shown in red. Dashed horizontal black lines highlight the fact that Rootine v.2 better captures root diameter in comparison with Rootine v.1.

Figures

Figure 1

Synoptic view of the Routine v.2 workflow including the comparison with Routine v.1.

Figure 2

Results of the steps of Routine v.2 for a subvolume of the worse case scenario; (a) The original greyscale image; (b) The obtained image after denoising with the 3D NLM filter; (c) The obtained image after performing edge enhancement of subfigure b; (d) Resulting image after background removal via an absolute difference transform on subfigure c; (e) Results of the root segmentation applied on subfigure d before applying postprocessing steps; (f) Segmented roots after applying the postprocessing steps on subfigure e.

Figure 3

Mask creation and calculation of the average root grey value based on characteristic peaks. Subfigure (a) depicts the drawing of a circular ROI bounded within the pot wall (red circle) on a 2D section of the worse case scenario. The bounded ROI serves the purpose of creating a mask. By extension of this bounded ROI by 50 pixels, an extended ROI is created (blue line); (b) Histogram of the bounded and extended ROI illustrated in subfigure a. The extended ROI serves the purpose of creating a peak in the histogram which will be used to calculate the average root grey value.

Figure 4

Estimation of σ values of the tubeness filters and the optimal lower thresholds of hysteresis thresholding. (a) Results of the tubeness filter on a hypothetical root of a diameter d_r obtained for different q values. The dashed blue lines show the original root outline whereas the solid yellow lines show the position of the transects used to plot the grey values along the root diameter axis; (b) Transects of the grey values along the root diameter axis for some of the q values shown in subfigure a. The color dots at the intersection between the root outline and the grey value parabola correspond to t_{hys}^{opt} for a given q value; (c) Line of best fit imposed on the couple of points q and t_{hys}^{opt} . In this study, we calculated t_{hys}^{opt} corresponding to $q = 0.5$ using the model regression. The calculated value is indicated by the dashed line (i.e. $t_{hys}^{opt}=79$).

	Minimum root diameter ↓	Root diameter increment = 4 voxels ↷					Maximum root diameter ↓
d_r [voxels]	4	8	12	16	20	24	28
d_r [μm]	180	360	540	720	900	1080	1260
σ	2	4	6	8	10	12	14
f_s	1	0.5	0.5	0.5	0.5	0.5	0.5
	Detect fine roots		Detect coarse roots				

Figure 5

Approach for the detection of roots of increasing diameters at the fine and coarse resolutions.

Figure 6

Vesselness score, Rb and Ra values for a sphere, a plate and a cylinder.

Figure 7

Illustration of the postprocessing steps implemented in Routine v.2. First, a 3D Median filter is applied on the results of the root segmentation step. Then, all connected objects are kept by applying the “Keep Largest” function. In order to ensure full connectivity of the roots at the top of the stack, a slice is added at the top (left-hand side of the figure). The remaining unconnected objects are subject to a test evaluating their shape, i.e. their “vesselness” and size. This is illustrated here by showing a Z-Projection of a 400x400x400 image from the best case scenario dataset (right-hand side of the figure). The green scale bar indicates the vesselness score whereas the red scale bar indicates the size score. The intensity of the yellow color depicts the combination of these two scores. If an object meets both the vesselness and size threshold, it is considered as a false negative and will subsequently be added to the connected root system. If not, it will be considered as a false positive and will thereafter be discarded.

Figure 8

Root recovery of Routine v.2 for the worse and best case scenario. (a&c) Comparison with the former Routine v.1 and the root length determined with destructive sampling and scanning of washed-off roots (WinRHIZO) for the worse case and the best case scenario respectively. The dashed line indicates the 1:1 line. (b&d) Visual comparison of the segmented root systems obtained with Routine v.1 and Routine v.2 for the corresponding sample circled in black on subfigures a and c for the worse case and the best case

scenario respectively. Roots detected by both algorithms are depicted in black, the ones only detected by Routine v.2 are shown in blue, whereas roots only detected by Routine v.1 are shown in red.

Figure 9

Root diameter distribution and root outline accuracy for the worse and best case scenario. (a&c) Root length density distribution as a function of root diameter for Routine v.1 and v.2 and the destructive sampling data obtained by scanning washed-off roots (WinRHIZO) for the worse case and the best case

scenario respectively. The semitransparent ribbon denotes the standard error of the measurements (n=12). (b&d) Visual comparison of the segmented root diameter outlines for both Routine v.1 and Routine v.2 supported by the original X-ray CT greyscale data for the worse case and the best case scenario respectively. Roots detected by both algorithms are depicted in black, the ones only detected by Routine v.2 are shown in blue, whereas roots only detected by Routine v.1 are shown in red. Dashed horizontal black lines highlight the fact that Routine v.2 better captures root diameter in comparison with Routine v.1.