3D micro–X-ray tomography of lumen space in dried stem, branch, and outer tissues of *Moringa oleifera*: A structural case study

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Abstract

Moringa oleifera is widely used in dry tropical and subtropical regions due to its rapid growth and high nutritional value, yet its internal tissue organization has primarily been described using two-dimensional anatomical approaches. Here, we present a three-dimensional micro–X-ray computed tomography (micro-XCT) characterization of lumen space in stem, branch, and outer tissues (bark region) from a single *M. oleifera*individual. Samples were oven-dried prior to imaging; therefore, the quantified void fraction represents apparent lumen/void space in dried material and should not be interpreted as in vivo porosity.

Micro-XCT datasets were segmented to quantify cross-sectional lumen area distributions and apparent void fraction from pooled reconstructed slices. All data originate from a single individual; reported metrics represent structural descriptors of pooled cross-sections and not replicated biological measurements. The stem dataset exhibited a dense arrangement of small lumen features and a high number of segmented objects, consistent with a compact woody tissue organization in the scanned region. The branch dataset showed a larger proportion of void space and a strongly right-skewed size distribution with a minority of large lumen features. The outer tissue dataset displayed heterogeneous void space organization, which likely reflects a mixture of cell lumens, intercellular spaces, and drying-related cracks, and therefore is reported descriptively without assigning xylem-vessel identity.

This study provides a conservative 3D structural dataset and an image-analysis workflow for quantifying lumen space in dried *M. oleifera* tissues, complementing published anatomical descriptions. The results highlight strong within-plant heterogeneity across tissue types and underscore the importance of sample preparation and histological validation when interpreting micro-XCT measurements in woody plants.

Highlights

• Micro-XCT provides 3D visualization and quantitative descriptors of lumen space in dried tissues.

• Stem, branch and outer tissues (bark region) show markedly different lumen space organization in the scanned regions.

• Lumen area distributions are strongly right-skewed, with many small features and a minority of larger features.

• Apparent void fraction measured from oven-dried samples is sensitive to preparation and should not be interpreted as in vivo porosity.

• The study provides a reproducible workflow for segmentation-based quantification of lumen space from pooled reconstructed slices.

1. Introduction

1.1. Ecological and applied relevance of Moringa oleifera

Moringa oleifera Lam. is a fast-growing deciduous tree often regarded as native to the Himalayan foothills and now widely cultivated throughout dry tropical and subtropical regions (Azmi et al. 2025). It is valued for its nutritional and agronomic relevance, particularly in regions with limited water availability and low-input agricultural systems. The leaves contain substantial amounts of minerals and β-carotene (a precursor of vitamin A), and the seeds and pods are used for food, oil production, and water clarification (Azmi et al. 2025). Due to its rapid growth and adaptability, *M. oleifera* has attracted attention as a multifunctional crop species in semi-arid environments (Parihar et al. 2022).

While the biochemical and agronomic properties of *M. oleifera* have been extensively investigated, its internal tissue organization has been primarily described using conventional two-dimensional anatomical approaches. Several studies have documented aspects of its wood anatomy, vessel size distribution, and scaling relationships along the stem (Olson 2001; Echeverría et al. 2019; Soriano et al. 2020). However, three-dimensional structural quantification of lumen space using volumetric imaging methods remains limited.

1.2. Wood anatomical background and structural context

In woody dicotyledonous species, the stem typically consists of secondary xylem (wood), secondary phloem, cambium, cortex, and periderm. The secondary xylem contains vessels that conduct water, along with fibers and axial and radial parenchyma. In contrast, the outer tissues (commonly referred to as bark) comprise secondary phloem and protective tissues and do not contain xylem vessels (Olson 2001; OLSON and CARLQUIST 2008).

Published anatomical studies of *M. oleifera* have described vessel diameter distributions (Echeverría et al. 2019; Soriano et al. 2020) and their variation along the axis of the plant. Vessel diameter and density are known to vary predictably with distance from the shoot apex in many woody species (Anfodillo et al. 2006; Fajardo et al. 2020) and *M. oleifera* follows similar axial scaling patterns (Soriano et al. 2020) (Lucas et al. 2013). Most of these analyses are based on histological sections, which provide high-resolution information in two dimensions but do not capture volumetric lumen connectivity or three-dimensional spatial organization. Unlike previous histological studies focused on vessel scaling and wood anatomy, the present work emphasizes volumetric characterization of lumen organization using micro-XCT.

A three-dimensional perspective may complement existing anatomical descriptions by enabling quantification of total lumen/void fraction and size distributions within defined regions of interest, provided that limitations of sample preparation are clearly acknowledged.

1.3. Micro–X-ray computed tomography in plant structural analysis

Micro–X-ray computed tomography (micro-XCT) enables non-destructive three-dimensional imaging of internal plant structures based on density contrast (Staedler Yannick M. AND Masson 2013) (Dhondt et al. 2010). In woody tissues, differences between cell walls and air-filled or low-density lumen spaces allow segmentation of void regions from surrounding material. Micro-XCT has been used in plant research to visualize structural organization and embolism formation (Brodersen et al. 2010) and to analyze tissue architecture in three dimensions (Staedler Yannick M. AND Masson 2013).

However, interpretation of micro-XCT data depends strongly on sample preparation. In dried samples, lumen dimensions may be altered by shrinkage, collapse of thin-walled cells, or formation of cracks. Therefore, quantitative descriptors derived from dried material represent apparent void space in the scanned state rather than in vivo anatomical dimensions (Piovesan et al. 2021).

1.4. Aim of the present study

The objective of this study was to provide a three-dimensional micro-XCT characterization of lumen space organization in stem, branch, and outer tissues (bark region) from a single mature *Moringa oleifera* individual. Using segmentation of reconstructed cross-sections, we quantified apparent void fraction and cross-sectional lumen area distributions in pooled slices for each tissue type. The aim was not to infer hydraulic performance, but to evaluate whether micro-XCT can provide reproducible three-dimensional descriptors of lumen organization in dried *Moringa oleifera* tissues.

The study is intended as a structural case study that complements published anatomical descriptions. No physiological measurements were performed, and the results are interpreted conservatively as descriptors of lumen organization in oven-dried material rather than as direct indicators of hydraulic function.

2. Materials and Methods

Plant material and sample preparation:

Stem, branch, and outer tissue (bark region) segments were obtained from a single mature *Moringa oleifera* individual cultivated in Brazil (Itabaiana, Sergipe). All scanned material originated from this same tree. The exact number of anatomical segments per tissue type was not recorded, as imaging was performed as part of an outsourced tomography campaign; however, all reconstructed datasets analyzed in this study correspond to material derived from this single individual.

Segments (approximately 5–10 cm in length prior to trimming for scanning) were oven-dried prior to micro-XCT imaging. No chemical fixation or embedding procedures were applied. Drying was performed to ensure dimensional stability during scanning. Because the material was oven-dried, the measured void fractions and lumen dimensions represent the structure in the dried state and may differ from hydrated in vivo anatomy due to potential shrinkage, cell collapse, or crack formation.

Micro–X-ray computed tomography (micro-XCT):

Tomographic imaging was conducted using laboratory micro-XCT systems (SkyScan 1272, Bruker, Belgium, and phoenix nanotom systems, Waygate Technologies, Germany). Imaging parameters were optimized separately for stem, branch, and outer tissue samples to achieve adequate contrast between cell walls and lumen space.

The scanning parameters for each tissue type are summarized in Table 1 (tube voltage, current, exposure time, voxel size, detector binning). Voxel size ranged from 5.00 µm to 6.25 µm depending on the scanning configuration.

Samples were mounted mechanically (stem and branch on a metal support with orthodontic wax; outer tissue inside a plastic tube with support material). Projection images were acquired over 360° rotation and reconstructed into two-dimensional cross-sections using filtered back-projection algorithms (phoenix datos|rec software) (Otsu 1979).

The resulting grayscale image stacks represent density contrast between solid cell wall material and lower-density lumen/void regions in dried tissue.

Table 1

Micro-XCT scanning parameters used for imaging *Moringa oleifera* bark, stem, and branch samples. The listed parameters include tube voltage, tube current, number of projections, pixel size, exposure time per projection, and detector binning, optimized for each tissue type to achieve high-resolution reconstruction. Voxel size differed between scanning setups: 5.00 µm for bark and branch datasets and 6.25 µm for stem datasets. Lumen areas were calculated directly from calibrated voxel dimensions. All lumen area and apparent void fraction calculations were based on voxel size–calibrated measurements for each dataset, so differences in voxel resolution (5.00 µm for bark/branch, 6.25 µm for stem) did not bias the results.

Tube voltage [kV]	**Bark**	**Stem**	**Branch**
	60	**60**	**60**
Tube current [µA]	200	200	150
Number of projections	1800	1800	1800
Pixel size [µm]	5.00	6.25	5.00
Exposure time / projection [ms]	1500	1500	750
Detector binning	1x1	1x1	2x2

Image processing and segmentation:

Image stacks were processed using ImageJ/Fiji (National Institutes of Health, USA). All slices were converted to 8-bit format and spatially calibrated using the voxel dimensions recorded during acquisition.

Segmentation of lumen/void regions was performed using threshold-based binarization. Otsu's method was used as an initial guide for threshold selection, followed by manual adjustment where necessary to ensure consistent separation between high-density cell wall regions and lower-density void space. Segmentation was performed by a single analyst to maintain internal consistency. Repeated trials on representative slices were conducted to ensure stable thresholding.

After binarization, connected components were identified using the "Analyze Particles" function in ImageJ. A minimum size threshold of 50 μm^2 was applied to exclude noise-level features. Circularity was not restricted, allowing detection of irregularly shaped lumen features.

For each detected object, cross-sectional area and centroid coordinates were recorded. All reconstructed slices for each tissue type were pooled prior to analysis. Therefore, the reported metrics represent descriptive statistics of pooled cross-sections from a single individual rather than replicated biological measurements.

Quantification of apparent void fraction and lumen area distribution:

Void fraction was calculated from binarized cross-sections as:

Apparent Void fraction = (total area of white pixels representing segmented void space) / (total cross-sectional area of the image)

Because samples were oven-dried, segmented white regions include:

- Xylem vessel lumens (in stem and branch)
- Parenchyma cell lumens
- Intercellular spaces
- Cavities present in the pith region
- Drying-related cracks

Accordingly, the term "void fraction" is used to describe the apparent proportion of low-density space in the dried sample rather than in vivo porosity.

For each segmented object, cross-sectional lumen area was computed from the number of pixels multiplied by pixel area (voxel size–calibrated). Distributions of lumen area were characterized using mean, median, and descriptive spread measures. Due to strong right-skewness in the distributions, median values are reported alongside means.

No statistical comparisons between tissue types were performed, as all data originate from a single individual and represent structurally distinct anatomical regions rather than biological replicates.

Limitations of sample preparation and biological replication:

All measurements were obtained from oven-dried material derived from a single *Moringa oleifera* individual. Drying may induce dimensional changes, including shrinkage of cell walls, collapse of thin-walled cells, and formation of cracks. Therefore, absolute lumen dimensions and void fractions should be interpreted as descriptors of the dried state.

Because only one individual was analyzed and cross-sections were pooled, the results do not capture inter-individual variability or axial variation along the stem. The study is thus presented as a structural case study intended to complement published anatomical literature rather than to provide species-wide quantitative generalization.

3. Results and Discussion

Stem (secondary xylem region)

Micro-XCT cross-sections of the stem revealed a concentric organization typical of woody dicotyledonous stems, consisting of a central pith region, a surrounding ring of secondary xylem, and outer tissues (Fig. 1). Within the secondary xylem region, numerous discrete lumen features, likely corresponding to vessel lumens were identified in cross-section. Many lumen features occurred in radial multiples, consistent with typical vessel grouping patterns in woody dicots.

The apparent void fraction of the entire stem cross-section in the dried state was 6.23%. A total of 23,757 lumen features were segmented across pooled reconstructed slices. The mean cross-sectional lumen area was 341.65 ± 10.10 μm² (standard error of the mean), and the median lumen area was 156.25 μm². The distribution was strongly right-skewed, with a large number of small lumen features and a minority of larger ones.

The central pith region exhibited higher void fraction relative to the denser secondary xylem region. These measurements represent structural descriptors of the dried material and do not capture axial variation along the stem or variability between individuals. Note that the pith cavity in *Moringa* is documented to contain lysigenous gum ducts rather than functioning as a water-conducting channel [6] (Olson 2002); the apparent void space in this region during micro-XCT scanning therefore likely reflects gum duct cavities and drying-related shrinkage rather than vascular function.

Branch

Micro-XCT cross-sections of the branch showed a central pith region surrounded by secondary xylem and outer tissues (Fig. 2). The general organization observed a central pith surrounded by secondary xylem and outer tissues, is consistent with the general stem structural plan described for *M. oleifera* and other *Moringa* species (OLSON and CARLQUIST 2008), including the presence of a pith containing lysigenous gum ducts. Within the xylem region, putative vessel lumens were detected among the segmented lumen features. Compared to the stem dataset, the branch dataset exhibited a larger apparent proportion of void space in the dried state.

The apparent void fraction for the scanned branch cross-sections was 90.80%, an unusually high value that likely reflects the combined effects of oven-drying, collapse of thin-walled tissues, threshold sensitivity during segmentation, inclusion of pith cavities, and drying-related crack formation, rather than in vivo porosity. Across pooled slices, 17,896 lumen features were identified. The mean cross-sectional lumen area was 6,616.81 ± 3,838.69 μm² (standard error of the mean), and the median lumen area was 1,875 μm². As in the stem dataset, the distribution was strongly right-skewed, with a small number of large lumen features contributing disproportionately to the mean.

Because the samples were oven-dried, the high apparent void fraction likely reflects not only vessel lumens but also lumen expansion, shrinkage-related deformation, and potential cracking in thin-walled tissues. Therefore, the reported values should be interpreted strictly as descriptors of the scanned dried state.

Outer tissues (bark region)

Longitudinal and cross-sectional scans of the outer tissues (bark region) revealed a heterogeneous distribution of void space (Fig. 3). Unlike the secondary xylem, the outer tissue region does not contain xylem vessels. The segmented void features in this region therefore represent a mixture of cell lumens (e.g., parenchyma or phloem-associated cells), intercellular spaces, and possible drying-induced cracks.

The apparent void fraction in the outer tissue dataset was 39.61%. A total of 11,342 void features were identified across pooled slices. The mean cross-sectional area of segmented void features was 4,551.14 ± 450.40 μm², and the median area was 1,875 μm². The distribution again showed strong right-skewness, indicating the presence of a minority of relatively large void regions within a background of smaller luminal spaces.

Given the lack of histological validation and the use of dried material, these void features are reported descriptively without assigning specific cell-type identity.

Comparative structural overview across tissues

Across the three scanned tissue types, marked differences in apparent void fraction and lumen size distributions were observed (Table 2). The stem dataset was characterized by a dense arrangement of numerous small lumen features and a relatively low apparent void fraction. The branch dataset exhibited the highest apparent void fraction and a broader size distribution including larger lumen features. The outer tissue dataset displayed intermediate apparent void fraction and heterogeneous void organization.

All reported values are based on pooled reconstructed slices from a single individual and therefore represent structural descriptors rather than statistically independent biological replicates.

Table 2
Quantitative structural descriptors derived from micro-XCT segmentation of dried *Moringa oleifera* tissues. Reported values include apparent void fraction, mean and median lumen area, and the number of segmented lumen features detected across pooled reconstructed slices for each tissue type. Because measurements were obtained from oven-dried material from a single individual, values represent descriptive structural metrics rather than replicated biological measurements. The identical median lumen area observed for branch and bark tissues (1,875 μm²) reflects the discrete pixel-area sampling intrinsic to the 5.00 μm voxel resolution used for these two datasets (yielding a per-pixel area of 25 μm²); both distributions independently converged on the same most frequent area bin rather than indicating a data-processing error.

Feature	Stem	Branch	Bark
Apparent void fraction (%)	6.23	90.80	39.61
Mean lumen area (μm²)	341.65 ± 10.10	6,616.81 ± 3,838.69	4,551.14 ± 450.40
Median lumen area (μm²)	156.25	1,875	1,875
Number of Segmented lumen features	23,757	17,896	11,342

4. Discussion

Structural characterization in the context of published anatomy

The present study provides a three-dimensional micro-XCT characterization of lumen/void space organization in stem, branch, and outer tissues of a single *Moringa oleifera* individual. Previous anatomical investigations of *M. oleifera* have described vessel diameter ranges, vessel grouping patterns, and axial scaling relationships using histological sections. The present volumetric approach complements these studies by quantifying apparent void fraction and lumen area distributions in three dimensions from pooled reconstructed slices.

Within the secondary xylem region of the stem and branch, segmented lumen features were consistent in size range and radial grouping with published anatomical descriptions of vessels in *M. oleifera*. The strong right-skewness of lumen area distributions observed here aligns with typical vessel size distributions reported in woody dicotyledonous species, where numerous small vessels coexist with fewer larger conduits. Because the present measurements were obtained from dried material and pooled cross-sections, they are interpreted descriptively and not as direct estimates of in vivo hydraulic dimensions.

The outer tissue (bark) dataset displayed heterogeneous void space organization. As expected for bark tissues, no xylem vessels are present in this region. The segmented void features likely correspond to a combination of parenchymatous cell lumens, phloem-associated structures, intercellular spaces, and drying-related cracks. Without histological validation, assignment of specific cell types is not possible. The outer tissue measurements are therefore reported as structural descriptors of the scanned dried state.

Influence of sample preparation on measured void fraction

Interpretation of micro-XCT data in woody plants depends strongly on sample preparation. In the present study, all samples were oven-dried prior to scanning and were not chemically fixed. Drying may cause shrinkage of cell walls, deformation of thin-walled tissues, collapse of parenchyma, and formation of cracks. Consequently, the measured apparent void fraction likely overestimates the fraction of lumen space present under hydrated conditions.

Methodological contribution of volumetric imaging

Despite these limitations, micro-XCT provides advantages for structural characterization that complement conventional histology. The technique allows non-destructive volumetric imaging and segmentation across entire cross-sections, enabling:

- Quantification of total void fraction over large areas
- Measurement of large numbers of lumen features across pooled slices
- Visualization of spatial heterogeneity within defined radial regions

It should be noted that the metrics reported here (apparent void fraction and cross-sectional lumen area) are two-dimensional, slice-wise descriptors obtained from a three-dimensional dataset, rather than measurements that are intrinsically volumetric (such as connectivity or tortuosity); as such, they could in principle also be obtained from conventional histological sections of equivalent quality.

The present workflow demonstrates that segmentation-based analysis of dried woody tissue can yield reproducible descriptors of lumen size distributions and apparent void organization, with the principal advantage over conventional histology being non-destructive access to complete, un-sectioned cross-sections at scale, rather than the extraction of metrics that are uniquely three-dimensional. Such descriptors may serve as a basis for future studies that combine volumetric imaging with controlled hydration states, histological validation, replicated sampling across individuals, or genuinely volumetric metrics such as lumen connectivity and tortuosity.

Scope and limitations

This study represents a structural case study of a single *Moringa oleifera* individual. All measurements were derived from pooled reconstructed slices and do not capture inter-individual variability, developmental gradients along the axis, or seasonal effects. It is also worth noting that *M. oleifera* has been characterized physiologically as exhibiting isohydric, water-saving behavior under drought stress rather than sustained high conductivity (Nardini et al. 2014); the present structural dataset does not address this physiological distinction and should not be read as implying otherwise.

The study is intended to provide a conservative three-dimensional structural dataset that complements existing anatomical literature.

Implications for future work

Future investigations could extend this approach by:

- Imaging hydrated or chemically fixed material to reduce shrinkage artifacts
- Combining micro-XCT with histological validation for cell-type identification
- Sampling multiple individuals to assess variability
- Standardizing measurements by distance from the apex to integrate with established scaling analyses

Such approaches would allow integration of volumetric structural analysis with established wood anatomical and physiological frameworks.

5. Conclusion

This study provides a three-dimensional micro–X-ray computed tomography (micro-XCT) characterization of lumen/void space organization in stem, branch, and outer tissues of a single oven-dried *Moringa oleifera* individual. Segmentation of reconstructed cross-sections enabled quantification of apparent void fraction and lumen area distributions across tissue types in the dried state.

Marked differences in structural organization were observed between secondary xylem regions and outer tissues. Lumen area distributions were strongly right-skewed, with numerous small features and fewer large void regions contributing to the overall structure. These results complement previously published two-dimensional anatomical descriptions by providing volumetric descriptors of lumen organization.

Because the study was conducted on oven-dried material from a single individual and no physiological measurements were performed, the findings are interpreted conservatively as structural descriptors rather than functional indicators. The presented workflow demonstrates the utility of micro-XCT for descriptive three-dimensional characterization of woody tissues and may serve as a foundation for future studies incorporating replication, histological validation, and controlled hydration states.

Abbreviations

Moringa oleifera (MO)
X
ray Computed Tomography (XCT)

Declarations

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Author contributions

Namrah Azmi and Jon Otto Fossum contributed to conceptual design. Material was sourced by Jon Otto Fossum, and preparations were performed by Namrah Azmi and Bruno Telli Ceccato. Data collection was performed by Heikki Suhonen and analysis was performed by Namrah Azmi. The first draft of the manuscript was written by Namrah Azmi and edited eventually by Bruno Telli Ceccato, Jon Otto Fossum. Feedback received on the draft from co-authors was added to the manuscript by Namrah Azmi and Jon Otto Fossum. All authors read and approved of the final manuscript.

Declaration statement:

The authors acknowledge the use of AI-based tools and automated image processing techniques, including Otsu's thresholding for binarization and automated segmentation methods for lumen detection and area quantification in XCT image analysis.

Conflict of Interest

The authors declare no conflict of interest.

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Figures

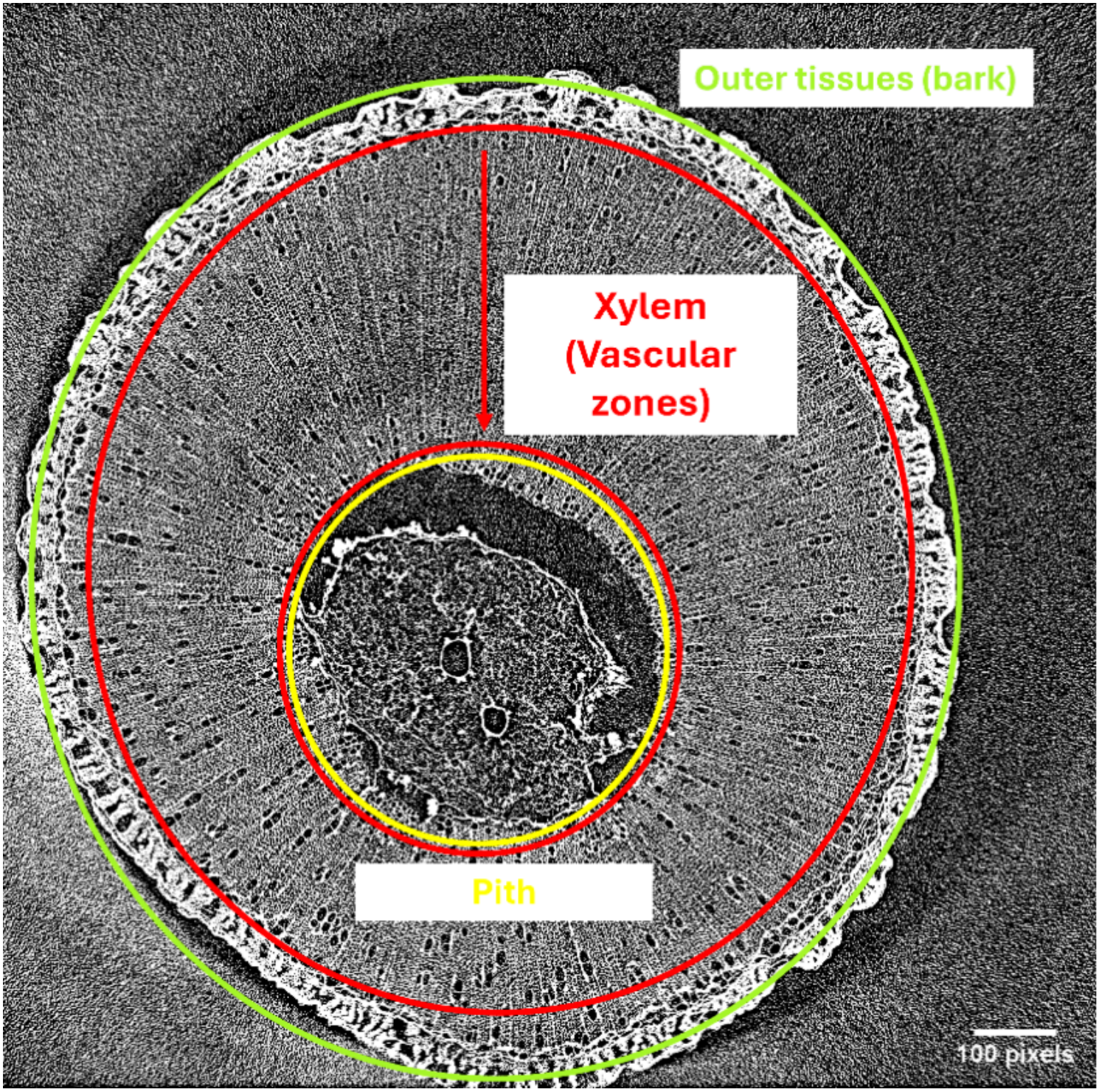

Figure 1

Representative micro–X-ray computed tomography (micro-XCT) cross-section of a dried *Moringa oleifera* stem. The image shows the central pith region, surrounding secondary xylem containing numerous vessel lumens visible in cross-section, and outer tissues. Segmented lumen features detected during image analysis are highlighted. The image illustrates the spatial organization of lumen space in the scanned region of the dried stem sample.

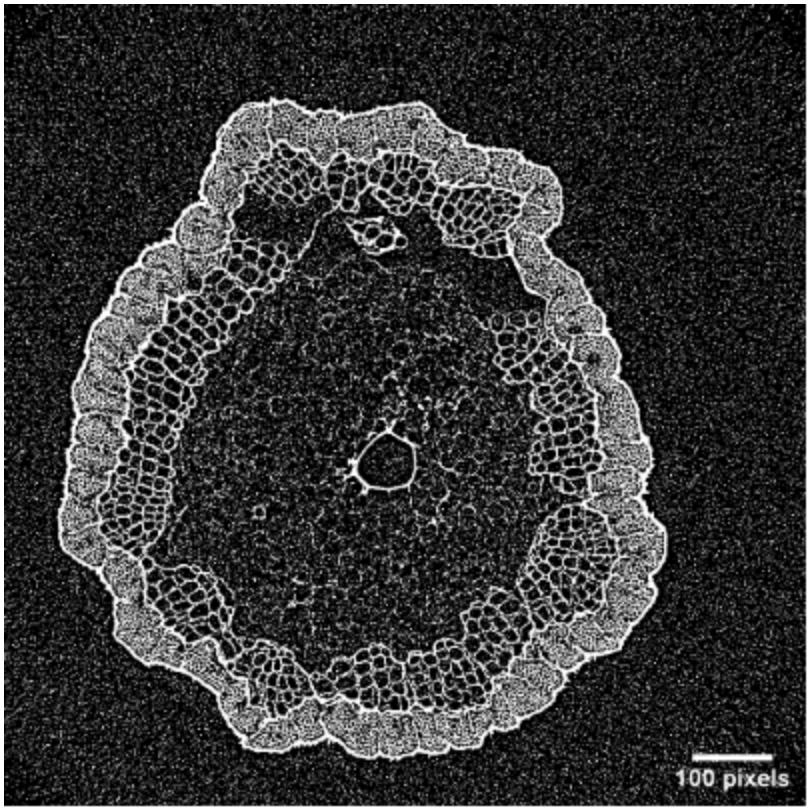

Figure 2

Representative micro-XCT cross-section of a dried *Moringa oleifera* branch. The branch cross-section shows a central pith region surrounded by secondary xylem and outer tissues. The image illustrates the spatial distribution and size variation of lumen features in the scanned region.

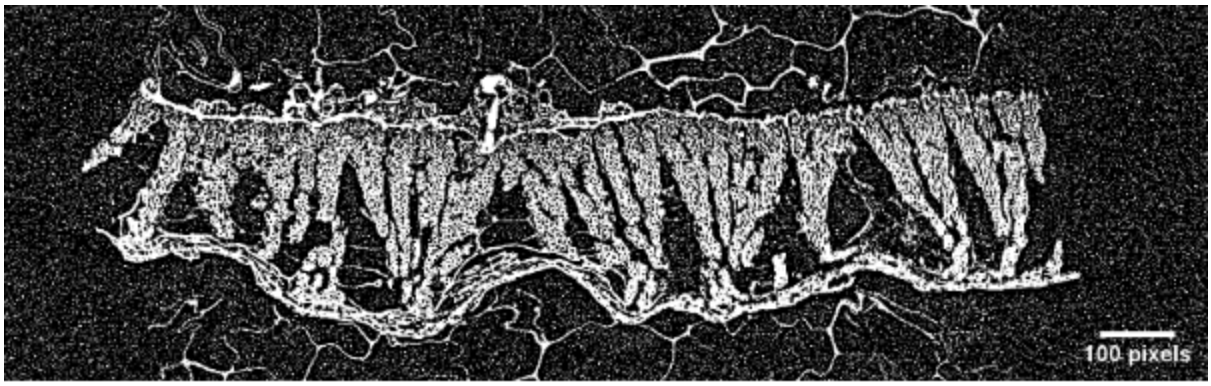

Figure 3

Longitudinal micro-XCT section of dried outer tissues (bark region) of *Moringa oleifera*. These void features represent a mixture of cellular lumens, intercellular spaces, and drying-related cavities. Because bark tissues do not contain xylem vessels, the segmented features are reported descriptively without assigning vessel identity.

Supplementary Files

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- floatimage1.jpeg