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# IDENTIFICATION OF WOOD AND CHARCOAL USING 3D-MICROSCOPY, µCT VOLUMETRIC IMAGING, AND MACHINE-LEARNING APPROACHES: A REVIEW

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## ABSTRACT

In an age of increasing surveillance and regulation of markets and trade flows, reliable methods for identifying the species and origin of commercial timbers and other woody plant products are essential. In highly processed and composite materials – such as charcoal, engineered wood products, and paper – DNA and chemical signatures are often degraded or modified. In contrast, anatomical structures are typically preserved, making structural identification the only reliable method. This review compares the principal anatomical methods used for wood and charcoal identification. It summarizes the strengths and limitations of traditional techniques and highlights recent advances in digital and AI-driven approaches, emphasizing innovations from the last ten years.

**Keywords:** wood identification, charcoal analysis, wood anatomy, artificial intelligence, digital image analysis, species identification, trade control

## INTRODUCTION

Wood and charcoal identification has a long and well-established research tradition (Figueiral and Mosbrugger, 2000; Gale and Cutler, 2000; Haag et al., 2017, 2022; Höhn and Neumann, 2012; Korte et al., 2024; Müller-Stoll, 1936; Di Pasquale et al., 2008; Scheel-Ybert, 2000; Schweingruber, 1978; Théry-Parisot et al., 2010; Zahnen et al., 2020; Zemke et al., 2025a, 2025b). Global wood-fuel production contributes substantially to forest degradation and deforestation, with far-reaching ecological consequences (Alkama and Cescatti, 2016; Betts et al., 2017; Chidumayo and Gumbo, 2010). This underscores the need for reliable identification tools in research, environmental monitoring, and trade control. Recent work by Steel et al. (2025) estimates global charcoal production at 70.5 million tons annually – about 50% higher than earlier estimates.

Over the past decade, major advances in digital microscopy, computed tomography, and related methods

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have greatly enhanced the quality and resolution of wood and charcoal identifications (Balzano et al., 2020; Bird et al., 2008; Braga et al., 2021; Van den Bulcke et al., 2009; Haag et al., 2020; Hubau et al., 2013; Nopens et al., 2025; da Silva et al., 2024; Stebani et al., 2025; Zemke et al., 2020, 2025). In contrast, genetic and chemotaxonomic techniques are unsuitable for charcoal, as carbonization destroys DNA and severely alters chemical signatures. Determining the geographic origin of charcoal using stable isotopes is also not currently viable. Charcoal's anatomical structure is inherently fragile due to carbonization, and archaeological materials are often further compromised by post-depositional microbial decay. As a result, producing thin microtome sections is impossible, and even stabilization attempts using paraffin, PEG, or resin have yielded unsatisfactory outcomes (Schweingruber, 1978, 2012).

Traditionally, charcoal analysis relied on reflected-light microscopy or standardized electron microscopy (EM). While both remain useful in certain contexts, they are less efficient than newer techniques. Reflected-light microscopy is limited by charcoal's deep black coloration, and different tissue types often cannot be distinguished clearly. This limits the ability to discriminate among very closely related species. EM provides high-resolution images but requires labor-intensive and costly sample preparation, making it impractical for routine use.

Three-dimensional reflected-light microscopy (3D-RLM) has emerged as a major step forward (Haag et al., 2018; Zemke et al., 2020). In this approach, irregular charcoal surfaces are digitally scanned to create 3D models, which are subsequently converted to high-quality 2D images. The use of polarization filters enhances contrast on dark surfaces, improving the visibility of anatomical tissues such as parenchyma, fibers, and other cellular structures (Fig. 1A). Zemke et al. (2020) demonstrated that 3D-RLM is approximately 20 times faster than EM. At the Thünen Centre of Competence on the Origin of Timber – a key European reference institution for wood identification serving testing authorities, market actors, and environmental organizations – about 20,000 individual charcoal fragments were examined and identified using this method between 2015 and 2025.

Computed tomography (CT) has been used for wood identification for nearly two decades (Van den Bulcke et al., 2009), particularly for the analysis of charcoal (Haag et al. 2022). Recent advances in µ and

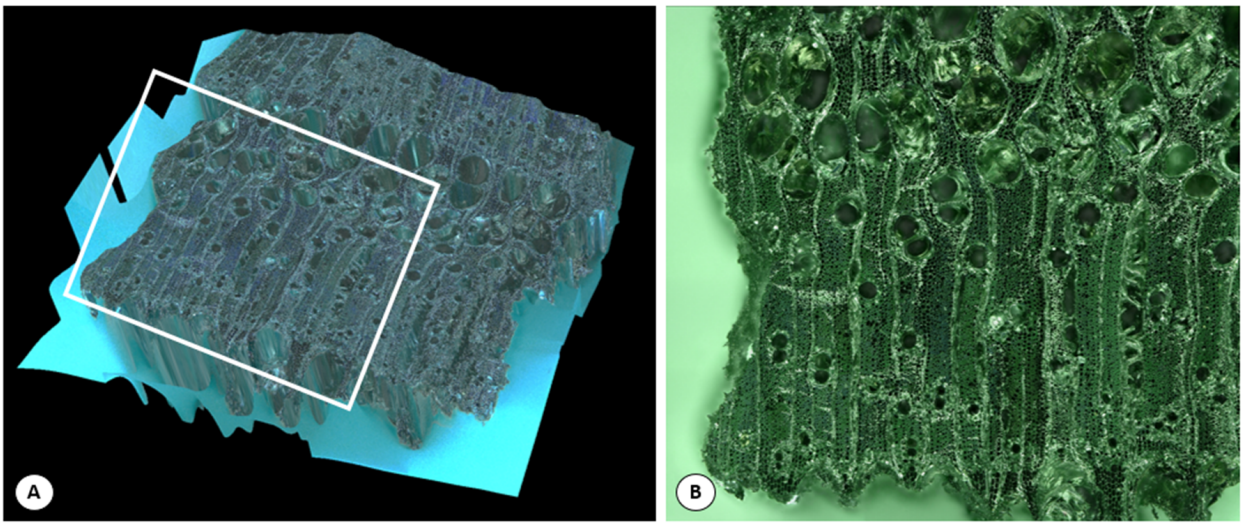


**Fig. 1.** *Fraxinus excelsior*: Three-dimensional representation of a charcoal fragment (left); the boxed area indicates the region converted into a two-dimensional image (right)
**Ryc. 1.** *Fraxinus excelsior* (jesion wyniosły): trójwymiarowa prezentacja fragmentu węgla drzewnego (po lewej); prostokąt wskazuje obszar przekształcony w obraz dwuwymiarowy (po prawej)

nano-CT have dramatically improved achievable resolution. CT offers clear advantages: it is fully non-destructive, requires no sample preparation, and allows the internal structure of fragile fragments to be examined as a complete volumetric dataset.

CT is now being integrated with other modern approaches. Stebani et al. (2025) demonstrated that CT-derived volumetric data can be used for automated wood identification using machine learning, reducing the need for specialized anatomical expertise. CT – particularly when combined with digital and AI-based techniques – is therefore regarded as one of the most promising technologies for future wood and charcoal identification.

The aim of this review is to compare the strengths and weaknesses of current methods and to assess which approaches hold the greatest potential in light of ongoing progress in digital and AI-driven technologies.

## METHODOLOGY

### 3D-reflected light microscopy (3D-RLM)

Microscopic examinations in Haag et al. (2017, 2020) and Zemke (2025a, 2025b) were performed using 3D-RLM to document diagnostic anatomical features. Digital image files were generated with a Keyence VHX-5000 digital microscope. Unlike solid wood, charcoal cannot be prepared with planar surfaces because charring causes extensive tissue degradation and extreme brittleness. To overcome this, 3D-RLM digitally scans the irregular fragment surfaces, producing three-dimensional models (Fig. 1A) that are subsequently converted into two-dimensional images for anatomical analysis (Fig. 1B). A detailed description of the method is presented in Zemke et al. (2020).

### Field emission scanning electron microscopy (FESEM)

In Zemke et al. (2020), charcoal fragments were examined first with 3D-RLM and subsequently with field-emission scanning electron microscopy (FESEM) to compare the total processing time from specimen preparation to image acquisition. For FESEM analysis, an FEI Quanta 250 FESEM was used. Specimens were trimmed to approximately 1 × 1 × 0.2 cm, surface-smoothed with razor blades, mounted on aluminum stubs with carbon paste, and sputter-coated with gold. Accelerating voltage ranged from 5 to 10 kV. Earlier studies (Blankenhorn et al., 1972; Cutter et al., 1980) report charcoal imaging without sputter-coating, whereas Gonçalves & Scheel-Ybert (2016) obtained better results using gold coating with a Bio-Rad SC 510 sputter coater. Platinum coating can also be used for enhanced resolution (Heu et al., 2019).

### µCT

The µCT analyses of Haag et al. (2022) were carried out at the Fraunhofer Development Center X-ray Technology (Würzburg). Most scans were performed using the "Click-CT", a prototype sub-micrometer, detector-based CT scanner optimized for organic materials. The system uses an optical microscope (detector) facing a scintillator plate that converts X-ray intensity from a micro-focal X-ray source into visible light. Specimens were positioned close to the detector to minimize penumbral blurring. Magnification was selected by using 4× and 20× objectives, yielding voxel sizes of 1.6µm and 0.3 µm, respectively; each specimen was scanned at both resolutions. The resulting volume images (2048 × 2048 × 2000 voxels in $x$, $y$, and $z$) amounted to roughly 16 Gigabytes per dataset (unsigned integer greyscale values). Scan times were 5 hours for 4× and 10 hours for 20× objectives (1800 projections, exposure times of 10 s and 20 s). All scans were conducted at 40kV and 200µA. For comparison, one *Alnus glutinosa* specimen was also scanned with the ntCT prototype, which achieves even higher resolution (voxel size 180 nm).

Volume rendering and anatomical feature extraction were performed using Avizo 9.04 (Thermo Fischer Inc.) and ImageJ2.

### AI / Deep Learning

Artificial intelligence (AI) and machine learning (ML) techniques offer transformative potential for automated wood analysis using µ and sub-µ-CT datasets, particularly for species identification and quantitative anatomical measurements. Modern systems may integrate deep learning architectures with traditional ML approaches to process high-resolution 3D tomographic data efficiently and accurately. Convolutional neural networks (CNNs) such as ResNet (Residual Network; He et al., 2016) can automatically learn hierarchical features from CT image slices, detecting complex

anatomical patterns – including vessel distribution, fiber wall thickness, and ray morphology – without manual feature engineering. Classification tasks can be performed using fully connected neural networks (FCNN; Rosenblatt, 1958), which operate on the extracted feature vectors to carry out binary or multiclass classification. U-Net architectures (Ronneberger et al., 2015) have proven particularly effective for semantic segmentation, enabling the delineation of vessels, fibers, parenchyma, and other cellular structures, and supporting precise quantification of morphological parameters such as porosity, cell wall–to–lumen ratios, and other three-dimensional structural metrics.

Training such models requires curated datasets of annotated CT images, with expert wood anatomists providing ground-truth species labels or voxel-level segmentation masks for cellular structures. Data augmentation procedures (e.g., rotation, scaling, and intensity variation) help expand limited training datasets while preserving realistic anatomical characteristics. Transfer learning and domain adaptation from pretrained models on related natural-image or microscopy datasets can substantially reduce training time and improve performance, particularly when fine-tuned on wood-specific features. Cross-validation protocols must account for biological variability within species and ensure generalization across growth conditions, preparation methods, and CT acquisition settings. Traditional ML methods, such as support vector machines, remain valuable when working with extracted feature sets, providing interpretable decision boundaries with careful feature selection and hyperparameter tuning.

Integrating these approaches yields comprehensive analysis pipelines in which deep learning models extract and quantify anatomical features, and classifier models separate species based on those measurements. Automated workflows can help to reduce observer bias, improve reproducibility across laboratories, and enable the detection of cryptic species or subtle morphological variations related to environmental stress or growth conditions – analyses that could be time-consuming and difficult to perform manually.

## RESULTS AND DISCUSSION

This section presents current examples that demonstrate (i) how microscopic technologies are already established in wood and charcoal identification, (ii) how these methods are currently applied in market monitoring, and (iii) which approaches offer the most promising prospects for future development. The study *The European Charcoal Trade* (Haag et al., 2020) illustrates the scale of charcoal that can be examined within defined timeframes, the insights achievable through large-scale anatomical analyses, and the policy-relevant conclusions derived from these examinations. The study *Volumetric Imaging by Micro Computed Tomography: A Suitable Tool for Wood Identification of Charcoal* (Haag et al., 2022) evaluates the suitability of high-resolution µCT, sub-µCT, and nano CT for wood identification and highlights the advantages of volumetric imaging over conventional microscopy. The study *Technical Framework of Advanced Volumetric sub-µ-CT Wood Imaging Integrated with Adaptable Deep-Learning-Based Wood Species Classification: Initial Evaluation on Softwood and Hardwood Data* (Stebani et al., 2025), represents the first global investigation integrating artificial intelligence with volumetric data for wood identification. The concluding summary synthesizes the methods applied across these studies and considers how these technological developments may influence future practices in wood and charcoal identification.

### The European Charcoal Trade

The study, *The European Charcoal Trade*, sought to provide a comprehensive pilot overview of the contemporary European charcoal market, including the range of timbers traded, the proportional use of subtropical and tropical species in the surveyed countries, and the accuracy of the accompanying documentation. The high-resolution analyses revealed that subtropical and tropical timbers accounted for approximately 46% of all material examined, with proportions exceeding 60% in consignments from Spain, Italy, Poland, and Belgium. A clear inverse relationship was observed between product certification (FSC and PEFC) and the presence of subtropical or tropical species in consignments collected in 2019–2020. For example, only 13.5% of the material from Switzerland originated from subtropical or tropical species, while 60% of products were certified. In contrast, consignments from Spain contained 67% subtropical or tropical timbers, yet only 8% carried certification. Examination of packaging and

the certificate declarations revealed substantial inconsistencies: only 25% of the consignments reported the wood species used, and more than half of these declarations were incorrect or incomplete. Owing to considerable public and scientific interest, the findings were highlighted in *Nature* Online News (Fig. 2).

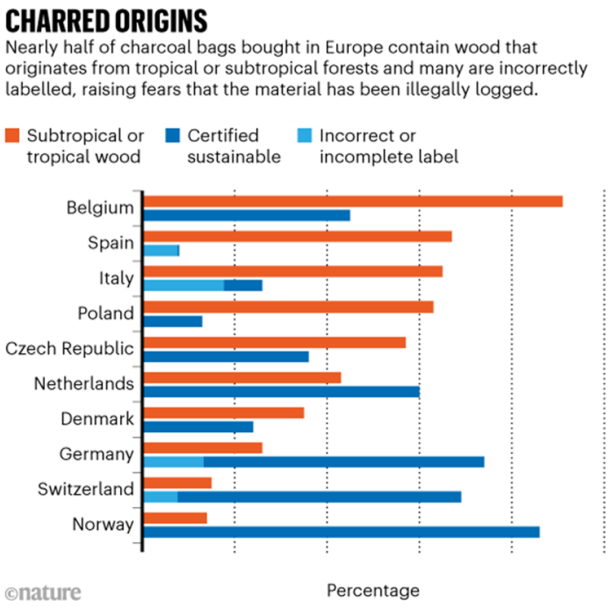


**Fig. 2.** Coverage of *The European Charcoal Trade* (Haag et al., 2020) in Irwin, 2020
**Ryc. 2.** Prezentacja wyników badań „The European Charcoal Trade" (Haag i in., 2020) w artykule Irwin, 2020

Approximately 150 samples of charcoal and briquettes were examined over a five-month period. At least 30 fragments per submission were analyzed, resulting in a dataset of roughly 4,500 fragments. The study generated significant public interest in Germany, Austria, and Switzerland and was widely reported and discussed in national media. Countries identified as critical – particularly Spain and Italy, where high proportions of subtropical and tropical species were detected – did not formally respond to the findings. Nevertheless, the results contributed to the inclusion of charcoal in the annex of the EU Regulation on Deforestation-Free Products (EUDR; Regulation EU 2023/1115). As a consequence, specific due diligence requirements will apply to charcoal from January 1, 2026 onward.

## Volumetric imaging by micro computed tomography: a suitable tool for wood identification of charcoal

The study first aimed to assess whether computed tomography (CT) data provide volumetric representations suitable for wood identification, using charcoal as a model material. Charcoal was selected because, unlike solid wood, it has a highly fragile and unstable structure, its chemical composition is altered, and its DNA is destroyed – conditions that render chemotaxonomic and genetic methods inapplicable. A second objective was to evaluate the resolution achievable with different CT systems and their adequacy for visualizing diagnostic anatomical features. To address these questions, scans were performed at three levels of resolution: µCT, sub-µCT (voxel sizes of approximately 1–5 µm), and nano CT (voxel size of 180 nm).

For a subsequent case study, three assortments of charcoal were purchased from different German supermarkets, representing a broad range of wood types and production conditions, including sustainability attributes. An initial random macroscopic examination was conducted, after which 30 fragments from each assortment were selected for CT scanning and documentation.

A key finding of the study was that the resolution required for accurate identification varies among wood species. Species such as oak (*Quercus* spp.) and ash (*Fraxinus* spp.) can often be identified macroscopically due to their distinctive anatomical features (Fig. 1), whereas closely related species – particularly those of tropical origin – or species lacking diagnostic structures require substantially higher resolution.

The results exceeded expectations, particularly with respect to the visualization of fine structural details. While most species could be identified at lower resolutions, the nano-CT enabled visualization of all diagnostic anatomical features, including extremely fine irregularities on cell walls that typically exceed what is needed for species identification. As shown in Figure 3A, structures such as scalariform perforation plates in alder (*Alnus glutinosa*) and other delicate anatomical features, including tyloses (Fig. 3B), were clearly resolved.

All charcoal fragments examined in this study could be reliably assigned to at least commercially relevant genera or anatomical groups (e.g., the "acacia

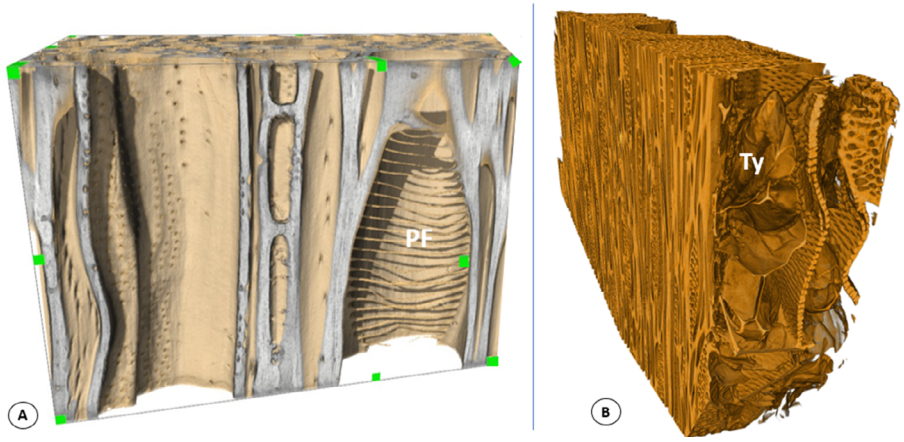


**Fig. 3.** Volumetric representation based on nano-CT measurement data: A – *Alnus glutinosa* showing scalariform perforation plates (PF), B – *Juglans regia* with tyloses (Ty) in vessel elements
**Ryc. 3.** Reprezentacja wolumetryczna oparta na danych pomiarowych nano CT: A – *Alnus glutinosa* (olsza czarna) z płytkami perforacyjnymi typu skalariformnego (PF), B – *Juglans regia* (orzech włoski) z tylozami (Ty) w elementach naczyniowych

type"). These results demonstrate that CT technology is well-suited for the identification of wood and charcoal. However, it must be emphasized that µCT technologies currently (as of 2025) remain significantly more expensive than conventional microscopy. Furthermore, the time required for individual processing steps – scanning, data reconstruction, and image conversion – is still considerably higher than for alternative techniques such as 3D-RLM.

Despite these limitations, CT provides two major advantages: (i) substantially higher achievable resolution and (ii) the ability to generate volumetric datasets that capture more structural information than a microscopic section or a surface-based 3D scan. Moreover, the large, information-rich datasets produced by CT are well-suited for advanced applications, including integration with artificial intelligence. These findings form the basis for additional research questions and subsequent projects, as outlined below.

## Technical framework of advanced volumetric sub-µ-CT wood imaging integrated with adaptable deep-learning-based wood species classification: initial evaluation of softwood and hardwood data

A recent study by Stebani et al. (2025) was the first to process volumetric (3D) sub-µCT data within a modular, multi-dimensional deep-learning and artificial intelligence framework for wood identification. Although this work used pristine samples of *Acer pseudoplatanus* and *Pinus sylvestris*, Haag et al. (2020) demonstrated that the approach can be directly transferred to processed wood products such as charcoal. Together, these studies underscore the potential of high-resolution X-ray tomography to discriminate wood species based on anatomical features – an essential capability for combating illegal timber trade, particularly where genetic or chemical methods fail due to material degradation. Imaging experiments were conducted using the sub-µ ClickCT system described by Zabler et al. (2020), configured for optimal contrast in low-Z organic materials such as carbon, oxygen, and hydrogen. Using a micro-focal source and 10× optical magnification, regions of interest (ROI) with an isotropic voxel size of 482 nm were acquired (Fig. 4). The resulting volumetric datasets provided excellent visualization of cellular features (e.g., fiber lumina, cell walls) and supercellular structures (e.g., vessels, rays) with qualitative fidelity comparable to stained cross-sections and the added benefit of ex post access to freely orientable slice images. For automated classification, a configurable processing pipeline based on residual networks (ResNet) was implemented as an open-source Python package, utilizing PyTorch (Paszke et al., 2019) as the deep learning backend. ResNets are

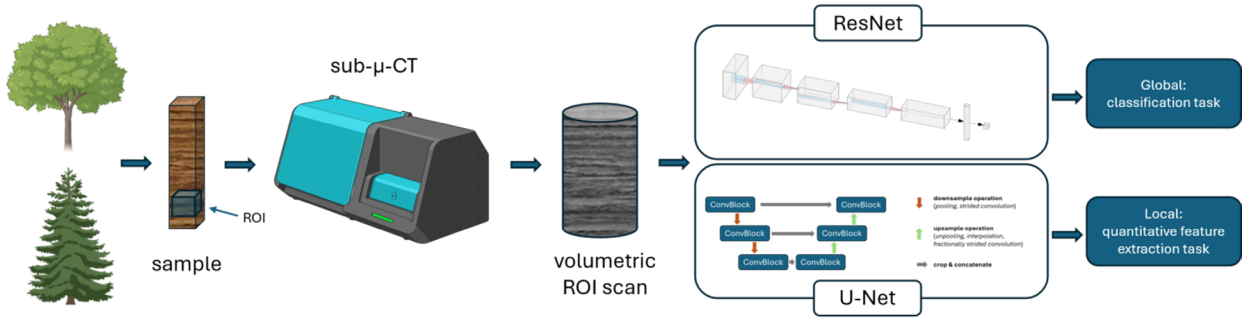


**Fig. 4.** Workflow from specimen to automated species classification or feature-based analysis in anatomical wood analysis. The workflow shown here focuses on global classification, additional quantitative information can be extracted in a similarly automated manner using related deep-learning architectures such as U-Nets, supported by the high image quality of the device (Created in BioRender; Stebani, 2025)

**Ryc. 4.** Aktualny schemat postępowania od obiektu do klasyfikacji lub analizy automatycznej opartej na cechach w kontekście anatomicznej analizy drewna. Choć opisywane badanie koncentruje się na globalnej klasyfikacji danych skanowania pod względem gatunku drewna, dodatkowe informacje ilościowe mogą być uzyskiwane w podobny sposób automatycznie przy użyciu pokrewnych metod głębokiego uczenia, takich jak U-Net, dzięki doskonałej jakości obrazowania urządzenia (opracowano w BioRender; Stebani, 2025)

composed of cascaded residual blocks that learn residual mappings, facilitating deeper architectures and stable gradient flow. Custom implementations were developed for 2D, 2.5D (concatenated orthogonal planes), and full 3D volumetric inputs. Numerical experiments compared these dimensional strategies within a unified framework. The initial evaluation demonstrated excellent classification performance for the exemplary binary species task. Robustness analyses showed substantial resilience to image degradation – including Gaussian, Poisson, and Gibbs noise – as well as resolution and geometric perturbations. These results establish volumetric sub-µ-CT as a strong foundation for large-scale wood-imaging databases that support machine-learning-based species identification, with clear implications for improved timber-trade monitoring and enforcement of forest-protection regulations.

The integrated framework unites the imaging pipeline with the multi-modal, multi-dimensional data-processing pipeline and has been successfully evaluated on representative hardwood and softwood datasets. This provides a robust basis for extending the approach to closely related species that are traditionally difficult to distinguish. The same datasets can also support feature-level analyses using U-Nets, enabling precise anatomical metrics (Fig. 3).

Beyond wood identification, the broader applicability of this framework to other organic materials with complex internal architectures represents an important next step. Non-destructive, rapid, and accurate identification of materials such as ivory, coral, or related biological substrates would significantly contribute to the enforcement of regulations such as CITES. Such applications underscore the wider scientific and societal relevance of combining high-resolution volumetric imaging with deep learning, positioning this approach as a pivotal tool for species conservation and legal compliance.

## CONCLUSIONS

The rapid advancement of digital technologies is driving continual improvements in established microscopic methods as well as the development of entirely new approaches. For example, three-dimensional reflected-light microscopy (3D-RLM) has substantially accelerated scanning and imaging workflows and enhanced the quality of anatomical information obtained from charcoal compared with conventional light microscopy. These technological advances have made it possible to conduct standardized, large-scale charcoal examinations – such as those routinely performed at the Thünen Centre of Competence on the Origin of Timber

in Hamburg for market monitoring and regulatory assessment. Such developments contribute significantly to investigations involving protected species under frameworks such as CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and regulatory measures, including the EU Regulation on Deforestation-Free Products (EUDR-2023/1115), which will introduce specific due diligence obligations for charcoal as of January 1, 2026.

Advances in computed tomographic methods show similarly promising potential. Although these approaches currently require substantial financial investment and offer comparatively low throughput due to the time-intensive measurement, reconstruction, and visualization steps, the resulting volumetric data provide considerable analytical advantages. This is particularly evident when such data are combined with artificial intelligence, such as machine learning methods for automated species identification. At present, only a few studies have explored this field, primarily as proof-of-concept investigations concerning wood. Nonetheless, the encouraging results suggest that similar approaches could be extended to other organic materials such as ivory or coral, supporting species conservation (CITES), or to forensic applications involving materials such as animal or human hair.

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## IDENTYFIKACJA GATUNKÓW DREWNA W WĘGLU DRZEWNYM ZA POMOCĄ MIKROSKOPII 3D I OBRAZOWANIA WOLUMETRYCZNEGO W POMIARACH µCT

### ABSTRAKT

W dobie rosnącego nadzoru i kontroli nad rynkami oraz przepływami handlowymi konieczne staje się opracowanie wiarygodnych metod identyfikacji gatunków i pochodzenia drewna handlowego oraz innych produktów pochodzenia drzewnego. W silnie przetworzonych i mieszanych materiałach – takich jak węgiel drzewny, materiały drewnopochodne czy papier – DNA oraz skład chemiczny ulegają degradacji lub zmianom, podczas gdy anatomiczna struktura drewna pozostaje często w pełni lub częściowo zachowana. Dlatego identyfikacja oparta na cechach strukturalnych stanowi jedyną możliwość. W artykule przedstawiono przegląd i porównanie głównych metod identyfikacji drewna i węgla drzewnego opartych na analizie anatomicznej. Podsumowano mocne i słabe strony tradycyjnych, ugruntowanych metod oraz podkreślono korzyści z zastosowania technik cyfrowych i metod opartych na sztucznej inteligencji, ze szczególnym uwzględnieniem ich rozwoju w ostatniej dekadzie.

**Słowa kluczowe:** identyfikacja drewna, analiza węgla drzewnego, anatomia drewna, sztuczna inteligencja, cyfrowa analiza obrazu, identyfikacja gatunków, kontrola handlu