Note: This is an accepted in-press pre-proof peer-reviewed draft of the journal article:

Kathryn E. Rankin, Zoë J. Hazell, Angela M. Middleton, Mark N. Mavrogordato (2021) "Micro-focus X-ray CT scanning of two rare wooden objects from the wreck of the London, and its application in heritage science and conservation". Journal of Archaeological Science: Reports 39, 103158

ISSN 2352-409X

DOI: https://doi.org/10.1016/j.jasrep.2021.103158

Available online 6th September 2021 at:
https://www.sciencedirect.com/science/article/pii/S2352409X21003709

Micro-focus X-ray CT scanning of two rare wooden objects from the wreck of the *London*, and its application in heritage science and conservation

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Abstract

Two wooden objects, a tuning peg from a stringed musical instrument and a stopper from a smoking pipe, were recovered from the AD 1665 wreck of the *London* and selected for wood identification. So far, they are the only recoveries of these object types from this wreck. To preserve their integrity and completeness, destructive sampling was not desirable. Instead, micro-focus computed tomography (µ-CT) scanning was carried out. The objects were scanned both pre-conservation (waterlogged/saturated) and post-conservation (PEG impregnated; freeze-dried). Although the aim was to non-destructively explore the internal structure of the objects for wood identifications, information was also gained on their manufacturing characteristics and internal condition. 1 µm voxel resolution – sufficient for positive identifications of these wood types to genus level (as is standard for wood identifications) – was achieved. This study has established that the conservation treatment used here does not obscure the microscopic anatomical features of these wood types and therefore recommends that µ-CT scanning is best undertaken after conservation, when the objects are stable.

Keywords

Micro CT scanning; archaeological conservation; archaeological artefacts; waterlogged wood; wood identification.

1 Introduction

The remains of the protected wreck of the *London* are located off Southend-on-Sea, Essex, UK, where they have rested since it sank following an explosion in AD 1665. In 2009 the wreck was put on the Heritage at Risk register (list entry 1000088) when it was recognised that it was at risk of loss through erosion. To mitigate this risk, a programme of surface recovery (by the licensed dive team) and limited excavations (led by Cotswold Archaeology, funded by Historic England) took place from 2014–2016. More than half of the recovered objects were wooden remains. These were complemented by glass, ceramic, metal and other organic (leather, rope) materials (see Walsh *forthcoming*). An extensive post-excavation phase – recording, analysing and conserving the objects – has since followed, with material analysis adding a valuable body of evidence to the archaeological record.

In heritage settings the need for sampling, especially invasive sampling, must be carefully considered because archaeological artefacts are unique and non-renewable. The removal of materials for analysis must be weighed up against information gain, and such complex decisions often involve several stakeholders. Guidance (Quye and Strlič 2019; *British Standard Institute* 2012) is available to assist in decision-making through a series of questions, including, for example: 'Have non-invasive options been explored fully?'

In the case of the wooden material from the *London*, one of the main research aims was to identify the types of wood (referred to here as 'wood types') used, by object category. In most cases this was possible using the standard wood identification technique of thin sectioning the wood to examine its microscopic anatomical structure (see Hazell and Aitken 2019). As a destructive technique, where possible, sub-samples were taken in already damaged or discreet areas.

However, options for studying rare and complete objects (here, a wooden pipe stopper and the tuning peg from a stringed instrument) required more-careful consideration. Options included i) no analysis, ii) invasive sampling and destructive analysis of the sample, and iii) non-invasive analysis. Option i) *no analysis* was discounted as the wood identification of these two rare examples from the archaeological context would add valuable historical information to our understanding of these object types. Option ii) was disregarded as both objects were completely preserved, and sample taking from already damaged or discreet areas was not achievable. Furthermore, any samples taken for thin-sectioning would be large relative to the small object size. As the priority for these two objects was to preserve them complete, option iii) *non-invasive analysis* was chosen.

A selection of non-destructive imaging techniques are available for use on wood, each with its own merits (often relating to the wood's preservation condition, be it waterlogged, desiccated, mineralised, or charred), and not all suitable for wood identifications (which depends on attaining spatial resolutions required to resolve wood cellular structure). These include:

i) Scanning Electron Microscopy (SEM); see Cartwight (2015)

SEM is a surface imaging technique requiring destructive sampling and therefore not suitable for the rare objects in this study.

ii) Magnetic Resonance Imaging (MRI); see Mori *et al* (2019) and Kanazawa *et al* (2017),

Based on the nuclear magnetic resonance of hydrogen, MRI is suitable for examining volumetric internal structure of waterlogged wooden objects, but is typically lower resolution compared to µ-CT (Mori *et al* 2019, Morales *et al* 2004, Kowalczuk *et al* 2019). Mori *et al* (2019) achieved 0.02 mm spatial resolution, which would not enable smaller features such as perforation plates, pitting and spiral thickenings to be resolved. Thus, MRI may be limited for the purpose of identification of wood anatomy.

iii) Neutron tomography (NT); see Lehmann *et al* (2005),

NT has high sensitivity for detection of hydrogen, suiting it to inspection and measurement of water and applied resin within wood. However, typical spatial resolution of NT (20 µm) would not be sufficient for identification of wood anatomy without diffraction measurement of neutron scatter (Heacock *et al* 2020).

iv) Synchrotron radiation computed tomography (SRCT); see Mannes *et al* (2010),

SRCT enables high resolution and high contrast volumetric imaging with high-brilliance X-rays, making it significantly faster than lab sources. It has been successful for multiple wood applications including anatomy identification for cultural heritage applications (Mizuno *et al* 2010, Tazuru and Sugiyama 2019).

v) Lab-based micro-focus X-ray computed tomography (µ-CT) (see below).

µ-CT is more readily accessible than SRCT, with image contrast primarily determined by variation in X-ray absorbance (Landis and Keane 2010). This may result in low contrast to noise ratio (CNR) between wood and water-saturated areas (O'Connor 2007, p. 46). Here, µ-CT was trialled as a non-destructive volumetric analysis tool to image the internal structure of the two objects. This technique has been successfully used on wood previously (Steppe *et al* 2004; Wei *et al* 2011) with multiple cultural and heritage applications for wooden objects, for example:

i) on individual musical instruments (Fioravanti *et al* 2017, Van den Bulcke *et al* 2017),
ii) on archaeological remains for wood identifications e.g. on wood charcoal (Bird *et al* 2008, Hubau *et al* 2013), on mineralised wood (Haneca *et al* 2012) and/or dendrochronological studies (Grabner *et al* 2009, Stelzner and Million 2015), and
iii) for experimental studies determining internal degradation e.g. fungal decay (Van den Bulcke *et al* 2008, Hervé *et al* 2014).

Prior µ-CT studies destructively sampled sections of wood for µ-CT. Here, µ-CT is conducted on complete objects in the waterlogged and conserved state for wood identification and internal integrity assessment.

2 The wooden objects

Following their recovery, the objects underwent desalination and conservation at Historic England's conservation facilities at Fort Cumberland, Portsmouth (Section 3.1). Two objects not to be destructively sampled were selected for µ-CT scanning to investigate their internal structure, primarily to determine the wood types:

- Pipe stopper (SF3342) (Figure 1A)
  A decorated stopper for use with a smoking pipe, probably used to tamp down tobacco. It is complete, but in two pieces: the head and the shaft. At its maxima, the head is 30.5 mm wide and 13.7 mm deep, and the shaft is 23.2 mm long, with 9.4 mm diameter at the lower end. When complete, it would have been approximately 56 mm tall.

- Tuning peg (SF3730) (Figure 1B)
  A tapered tuning peg from a stringed musical instrument: 59.2 mm total length and 14.8 mm wide at the head (the finger grip). The shaft is conical, 39.7 mm long, with a taper of 2.6 degrees and a hole (1.7 mm diameter) for the string, centred approximately 17.0 mm from the tip. On close examination whilst still waterlogged, a circular insert (2.6 mm diameter at the surface) of a different, paler-coloured unknown material was observed on the top edge of the head, likely as decoration.

(Reported dimensions from µ-CT data of the conserved objects (see Section 3.4.4)).

Figure 1. Photographs (post-conservation) of (A) the pipe stopper, showing the head section at the top left and the shaft section at the bottom right of the image, and (B) the tuning peg, with the head section (finger grip) on the left and the shaft section on the right of the image.

2.1 Decay and conservation treatments of waterlogged wood

As soon as it enters the ground or the water, wood, a natural material, is subject to biodegradation. Compared to terrestrial sites, wood is normally better preserved in waterlogged environments, due to lower oxygen levels which slow down fungal and bacterial decay processes. Wood decay in wet/waterlogged environments, as in the case of the *London*, is mainly caused by soft rot fungi, and/or by tunnelling-, erosion- or cavity-bacteria (Hocker 2018, p. 13, Hoffman 2013, p. 26). Microorganisms preferentially degrade the cellulose rich secondary layers of the wood cell wall, leaving the lignin rich middle lamella behind. Additionally, in the marine environment marine wood borers (*Teredo* and *Limnoria* species) burrow channels into the wood. Decay starts on the outside and over time moves into the centre of the wood, resulting in a more decayed outer layer and better-preserved inner section (Hoffman 2013, p. 27). Both micro- and macroscopic damage removes wood substance, resulting in a physically weakened and soft material. Gaps created in the cell wall due to microscopic decay are filled with water, which supports the weakened wood cell structure and at the same time preserves the wood's overall shape and dimension.

As a result of material loss, waterlogged archaeological wood can rarely be dried without a pre-treatment (Hocker 2018, p. 69) and requires a more interventive approach. All wood conservation techniques have the same overall goal: to remove water from within the wood cell structure whilst limiting dimensional changes. This is normally achieved by impregnation followed by drying. A number of materials have been used for impregnation, such as sugars, or synthetic materials such as Kauramin (a melamine resin) or PEG (polyethylene glycol). Drying methods can include slow air-drying or vacuum freeze-drying. It is worthwhile mentioning that some dimensional changes are inevitable as wood transitions from waterlogged to dry.

The conservation methods selected here were impregnation with PEG followed by vacuum freeze-drying, which is a well-established and commonly-used combination approach. Examples include small finds from the shipwrecks of the *Vasa* and *Mary Rose* (Hocker 2018, p. 69; Jones 2003, p. 64). PEG fulfils the role of a bulking agent by providing support to the wood cell structure, ensuring that the overall shape and dimensions are preserved. PEG is available in different grades, ranging from liquid to solid. Using two different grades (liquid/ low and solid/ high molecular PEG) provides support through bulking across well- and less well-preserved areas of wood, respectively (Hoffman 2013, p. 59). PEG also has very good aging properties (Jones 2003, p. 64). Vacuum freeze-drying is a slow and gentle drying technique which avoids the liquid phase of water and thereby circumvents surface tension of water when it evaporates (Flink and Knudsen 1983), which would be strong enough to cause cell collapse (shrinking, splitting and warping) if archaeological wood were to air-dry (Barbour 1990, p. 187).

3 Methods

3.1 Conservation of the pipe stopper and the tuning peg

First, the objects underwent desalination to remove soluble salts. This involved frequent water changes using distilled water and checking the conductivity of the storage water (Cronyn 1990, p. 81). Once the conductivity had plateaued, active conservation was implemented. Prior to PEG treatment, the objects were µ-CT scanned (Section 3.3).

Wooden objects were submerged in 30% PEG 400 (low molecular) (volume by volume) for three months and 30% PEG 4000 (high molecular) (weight by volume) for another three months. They were then pre-frozen at -30°C in a domestic chest freezer for one week, before being freeze-dried in a LyoDry Midi Freeze Dryer s/n F012. The chamber temperature was set at -30°C and the condenser temperature was set to -45°C. The removal of water via sublimation during the vacuum freeze-drying process was monitored by weighing the objects. The endpoint was determined when the weight-loss plateaued. Excess PEG was carefully removed from the wood surface after freeze-drying, using brushes and wooden skewers. The objects were then CT-scanned (Section 3.4).

3.2 µ-CT Scanners

Lab µ-CT scanner selection was first governed by scan time criteria, set for the waterlogged objects to keep scans as short as possible to minimise drying. The first aim was to obtain the full geometry (‘overview’) of the object in one scan, at the highest resolution possible i.e. filling the field of view (FOV). For these faster, lower resolution overview scans, a modified 225 kVp (225 W) Nikon/X-tek HMX µ-CT scanner (Nikon metrology, UK) (‘HMX’) with a Perkin Elmer XRD 1621 CN14 HS detector (PerkinElmer Optoelectronics, Germany) and Tungsten reflection target material was selected. This scanner is capable of a range of spatial resolutions ranging from 3 microns (6 mm FOV) to 55 microns (110 mm FOV).

Once the objects were conserved and dried, the time criteria were removed enabling longer scan times for identification of micron-scale anatomical features within a region of interest (ROI) at higher spatial resolution. A 160 kVp (10 W) Zeiss Xradia Versa 510 X-ray microscope CT scanner (Carl Zeiss Microscopy GmbH, Germany) (‘Versa’) with a transmission target was selected. This scanner is capable of sub-micron (~600 nm) spatial resolution with two stage magnification; primary magnification is geometrical from the X-ray cone beam and source-to-object/source-to-detector distances (SOD/SDD), and secondary magnification occurs post-scintillation as optical microscope objective lenses further magnify the image ahead of the charged-coupled device (CCD) detector (Appendix Figure A1).

3.3 μ-CT of waterlogged wooden objects (pre-conservation)

Each object was removed from water prior to scanning and mounted in polymer foam within a polymer beaker, sealed with Parafilm™ to minimise drying.

3.3.1 Pipe stopper

The pipe stopper was scanned in the HMX at 100 kVp peak voltage and 40 W power, with an SOD of 88.4 mm and an SDD of 755.0 mm. Using an analogue gain of 24 dB and binning 1 of the detector, 2801 and 1601 projection images were acquired throughout 360 degrees rotation of the head and shaft pipe stopper sections, respectively, averaging 8 frames per projection (FPP) with 134 ms exposure time per frame. The head and shaft sections were scanned separately to maximise the voxel (volume element or cubic pixel) resolution achievable.

3.3.2 Tuning peg

The tuning peg was scanned in the HMX at 80 kVp and 45 W, with an SOD of 127.0 mm and an SDD of 704.0 mm. With 18 dB analogue gain and binning 1, 1201 projection images were acquired, averaging 4 FPP with 134 ms exposures.

3.3.3 Data preparation

Projection images from the HMX were reconstructed into 32 bit float volumetric datasets using filtered back-projection algorithms implemented within CTPro3D and CTAgent software v2.2 (Nikon Metrology, UK). The resulting voxel resolution was 23.4 μm and 36.1 μm for the pipe stopper and tuning peg, respectively. Each 32 bit raw volume was down-sampled to 8 bit using ImageJ/Fiji (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2019) to reduce data processing time.

3.4 μ-CT of conserved wooden objects (post-conservation)

The conserved objects were scanned at higher resolution using the Versa scanner, with the aim of making specific identifications of wood type by achieving the best spatial resolution possible (sub-micron).

3.4.1 Pipe Stopper

The shaft of the pipe stopper was selected for this scanning phase, to minimise the X-ray penetration path length and thus maximise the possible signal-to-noise ratio (SNR). The shaft was wrapped in Parafilm™ and mounted in a thin-walled polymer tube on an aluminium base. A two stage approach first located an ROI within the shaft (i.e. in a representative area which was not cracked or excessively degraded) (Stage 1) and then scanned at sub-micron resolution (Stage 2), using parameters outlined in Table 1. The voxel resolutions achieved in the first and second scan stages were 3.0 μm and 0.7 μm from approximately 2.5 hours and 75 hours acquisition time, respectively.

Object	Scan Stage	Peak Voltage (kV)	Power (W)	SOD (mm)	SDD (mm)	Obj.	Bin.	Exp. (s)	Proj. / FPP	Voxel Resolution (µm)
Pipe Stopper	1	80	7	16.1	36.5	4X	2	1	3201 / 1	3.0
	2	80	7	16.1	30.5	20X	2	32	8001 /1	0.7
Tuning Peg	0	80	7	16.1	136.1	0.4X	2	1	801 / 1	8.2
	1	80	7	16.1	36.5	4X	2	1	3201 / 1	3.0
	2	80	7	16.1	30.5	20X	2	32	8001 / 1	0.7
	3	80	7	16.9	108.1	4X	2	5	6001 / 1	1.0

Table 1. The µ-CT parameters used for scanning the conserved pipe stopper shaft and tuning peg. SOD = source-to-object distance, SDD = source-to-detector distance, Obj. = magnification objective, Bin. = binning, Exp. = exposure time, Proj. = number of projection images, and FPP = frames per projection.

3.4.2 Tuning Peg

The tuning peg was mounted in the same tube as the pipe stopper, but with the addition of low-density floral foam at the base and a collar of Parafilm™ at the top of the tube, to minimise movement. A multi-stage scanning approach (Table 1) was used to first locate the circular insert ROI and a secondary ROI within the main body of the peg itself, at lower resolution (8.2 µm voxel resolution) (Stage 0), before following the protocol used to scan the pipe stopper (Stages 1 and 2). An additional scan (Stage 3) was performed at 1.0 µm voxel resolution, as a compromise in resolution, FOV, and exposure time, which could be reduced due to the increase in flux with the 4X objective, thus reducing scan time (to approximately 11 hours).

3.4.3 Data preparation

The projection data from the Versa was reconstructed using the Zeiss XM Reconstructor software (Carl Zeiss Microscopy GmbH, Germany) into 16 bit TXM files then converted to 16 bit raw volumes.

3.4.4 µ-CT overview scans of conserved objects

Finally, the objects were also scanned in the HMX scanner again to evaluate the full geometry once conserved using the parameters in Table 2. The head and shaft of the pipe stopper were scanned individually, and four scans were acquired to cover the overall height of the tuning peg at higher resolution. The same reconstruction method was used as in Section 3.3.3, to give 8 bit raw volumes, which were then concatenated.

Object	Peak Voltage (kV)	Power (W)	SOD (mm)	SDD (mm)	Analogue Gain (dB)	Bin.	Exp. (ms)	Proj. / FPP	Voxel Resolution (µm)
Pipe Stopper head	80	28	83.2	749.5	24	1	250	2801 / 8	22.2
Pipe Stopper shaft	80	28	83.2	749.5	24	1	250	1201 / 8	22.2
Tuning Peg	80	20	39.3	797.9	24	1	354	1501 / 8	9.9

Table 2. The µ-CT parameters used for scanning the conserved objects within the HMX to evaluate full geometry.

3.5 Wood identifications

The wood was identified using the identification texts and keys by Schweingruber (1990), Schoch *et al* (2004) and Gale and Cutler (2000). Standard identifications involve recording features seen on the three planes: transverse section (TS), radial longitudinal section (RLS) and tangential longitudinal section (TLS).

Identification attempts were made using the pre- and post-conservation µ-CT volumes. Each raw volume was imported into VGStudioMax v2.1 (Volume Graphics GmbH, Germany) with an *xyz* co-ordinate system. The volumes were reorientated to align the orthogonal planes (*xy*, *xz* and *yz*) with the TS, RLS, and TLS planes so that they could be inspected for microscopic wood anatomical features. To achieve secure identifications, it was necessary to locate key small characteristics such as: ray width and ray cell type, perforation plates and spiral thickenings. In the case of the perforation plates, the volumes had to be further re-orientated to see them in plane (Figure 6). Typically, this meant tilting by 30–45º (relative to each vessel's axis).

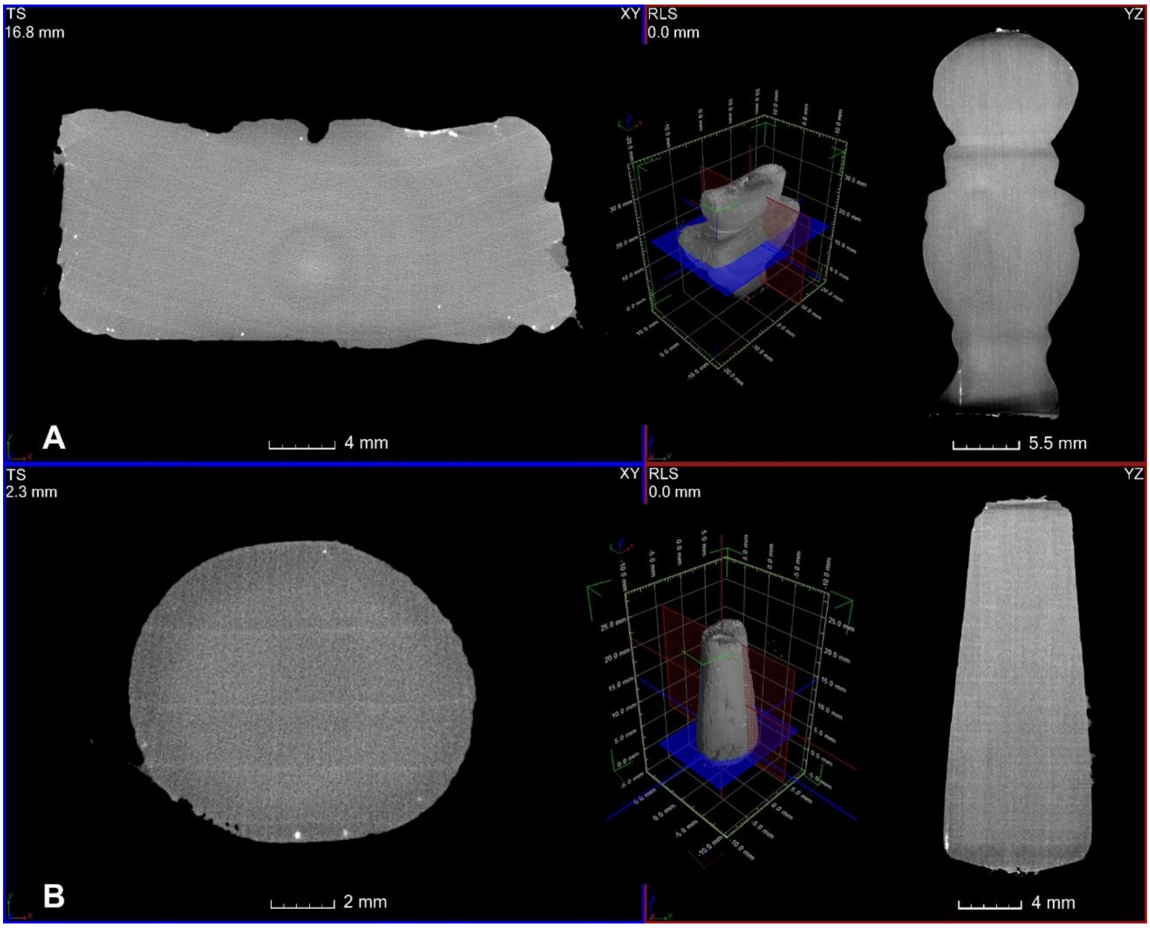
4 Results and Discussion

4.1 Pre-conservation

4.1.1 Pipe stopper

The results of the pre-conservation µ-CT of the pipe stopper are illustrated in Figure 2 (with head 3D rendering in Appendix Figure A2). The concentric rings are a CT artefact, known as ring artefacts (Barrett and Keat 2004) and are more visible post-conservation (Figure 13). These originate from consistent false signals from some pixels on the detector that, when reconstructed from 360 degrees of projection data, become a ring in the plane perpendicular to the axis of rotation.

Figure 2. Slice data through the TS (blue plane) and RLS (red plane) of the pipe stopper, before conservation (HMX): (A) upper piece (head), and (B) lower piece (shaft).

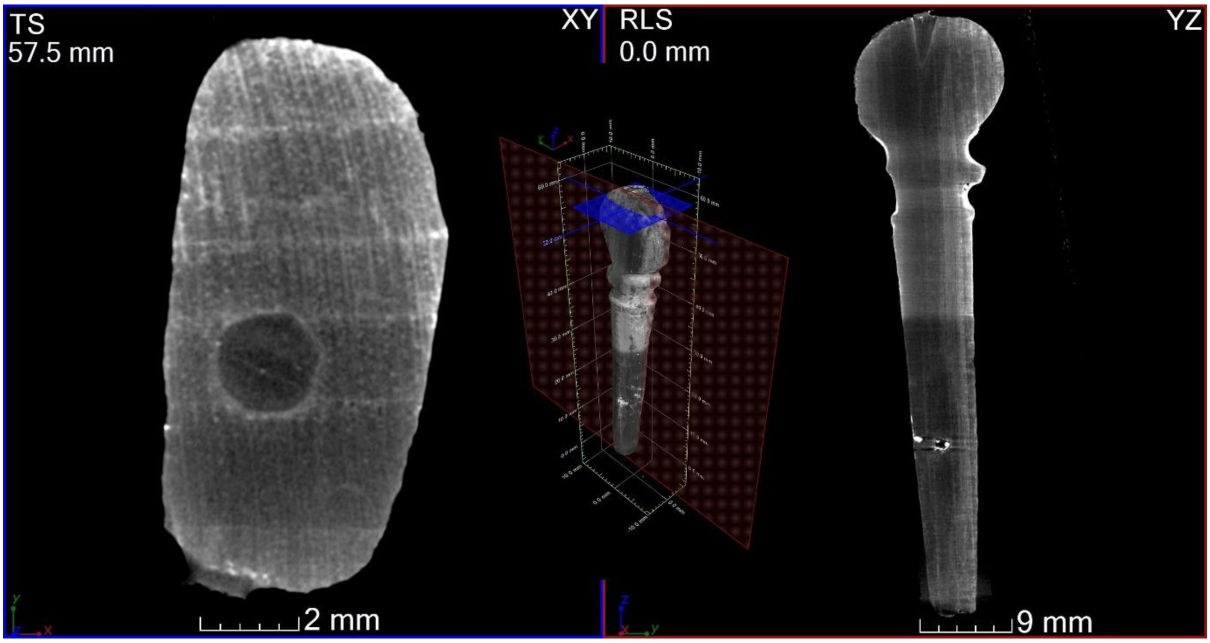


On the pre-conservation (wet/waterlogged) µ-CT scans it was possible to identify growth ring boundaries. On the head of the pipe stopper, the ring boundaries were aligned approximately parallel with the longest face of the object (Figure 2(A)). Whilst the structural elements required for a secure wood identification were not adequately resolved due to low CNR, it was possible to identify it as an angiosperm (hardwood) and discount certain distinctive wood types with larger, characteristic features (e.g. those with a ring porous vessel structure such as *Quercus* sp. (oak)).

4.1.2 Tuning peg

The results of the pre-conservation scanning of the tuning peg are presented in Figure 3 (with 3D rendering in Appendix Figure A3). It can be seen that the circular insert on the top edge of the head (finger grip) was in fact a conical insert set 5 mm into the main body of the tuning peg.

Figure 3. Slice data through the TS (blue plane) and RLS (red plane) of the tuning peg, before conservation (HMX).



On the body of the tuning peg it was possible to resolve growth rings, but the exact number of rings was uncertain due to limited image contrast. Overall, the wood looked to be an angiosperm (hardwood), with faint suggestion that the rays could be multiseriate. On the head section (the finger grip) the growth ring boundaries were aligned perpendicular to its longest edge. It was also just possible to distinguish growth rings on the decorative cone insert confirming it to be wood rather than another material (e.g. shell/bone).

4.2 Post-conservation

The scans on the Versa achieved sub-micron spatial resolution, such that the wood anatomical features required for a secure identification were visible.

4.2.1 Pipe stopper

The wood is identified as a *Betula* sp. (birch), from the combination of the following features: i) diffuse porous alignment of vessels with short radial chains (Figures 4 and 5 (TS)), ii) the presence of scalariform perforation plates generally with more than 10 narrowly-spaced bars (Figure 6), iii) multiseriate rays (Figure 5) and iv) absence of aggregate rays. Additionally, although the individual vessel pits (~1 µm) were not fully resolved, their alignment which formed an overall ‘fingerprint’-like pattern was seen (Figure 5); this is a characteristic frequently seen by the specialist (ZJH) in other material of this wood type.

From the post conservation ‘overview’ µ-CT volume on the HMX, it was possible to clearly identify and distinguish growth rings in the head section of the pipe stopper.

Figure 4. Post conservation (Versa) pipe stopper Stage 1: Slices through the TS shows a growth ring boundary and vessels with short radial chains, but in the TLS, 3 µm voxel resolution was insufficient to resolve the detail of the ray cells.

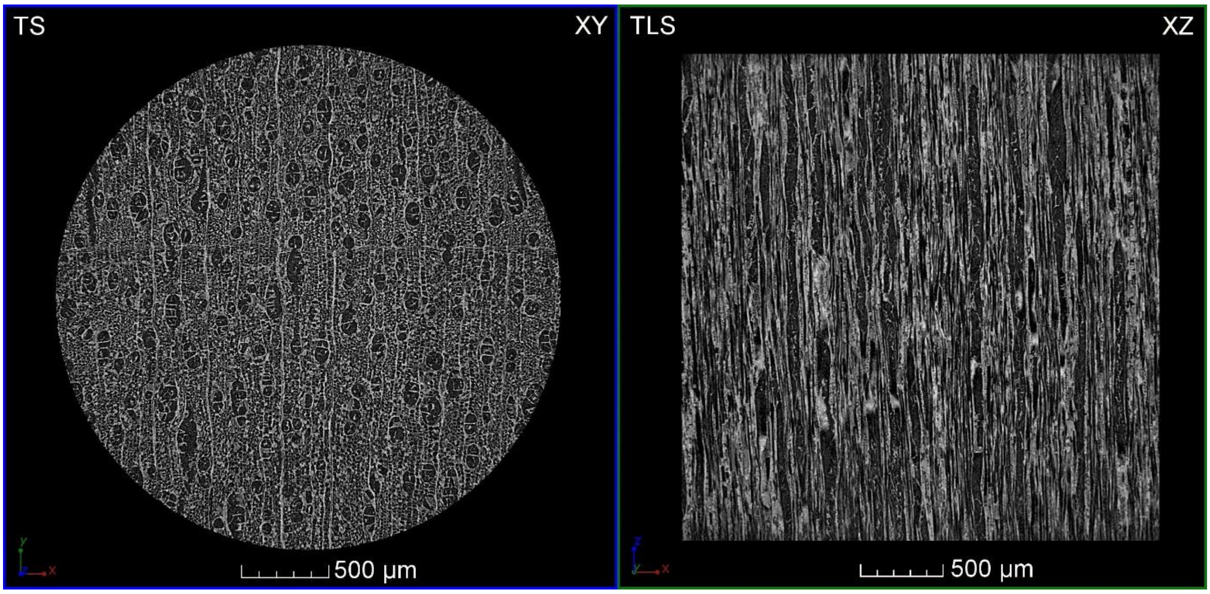


Figure 5. Post conservation (Versa) pipe stopper Stage 2: Slices through the volume at 700nm voxel resolution show short radial chains (TS), multiseriate rays (TLS) and fingerprint pattern (TLS).

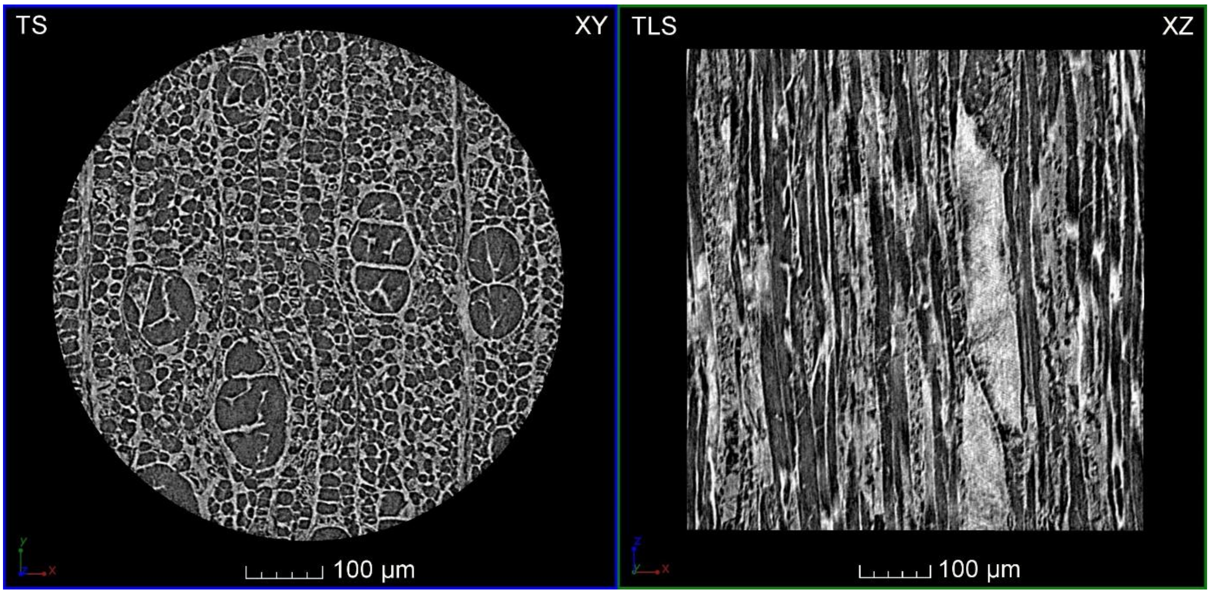
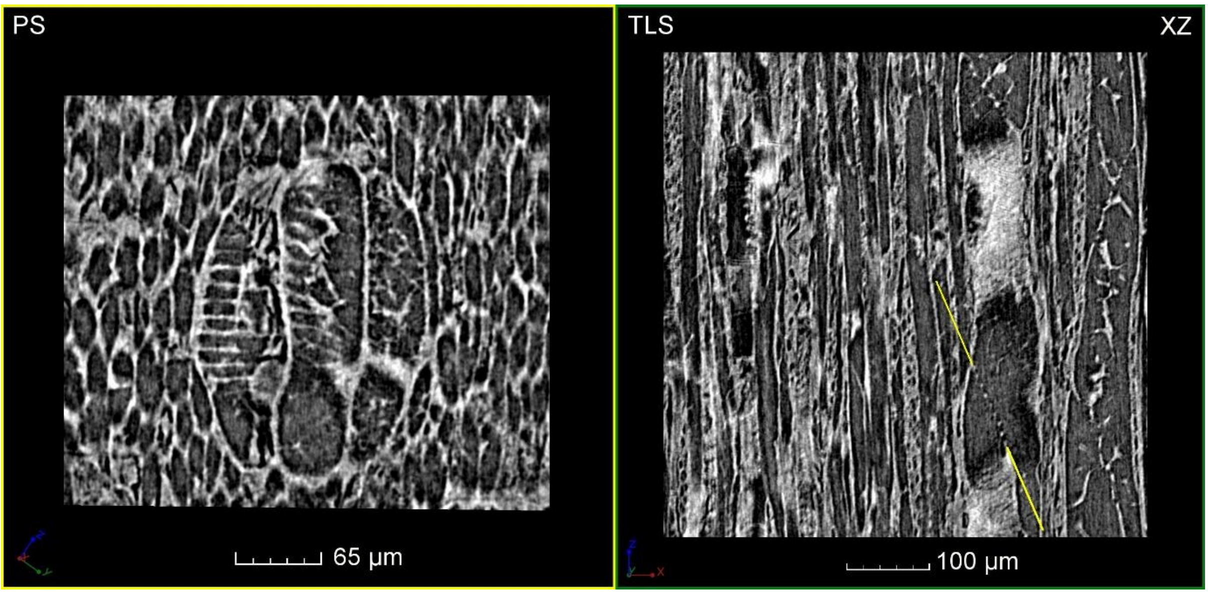


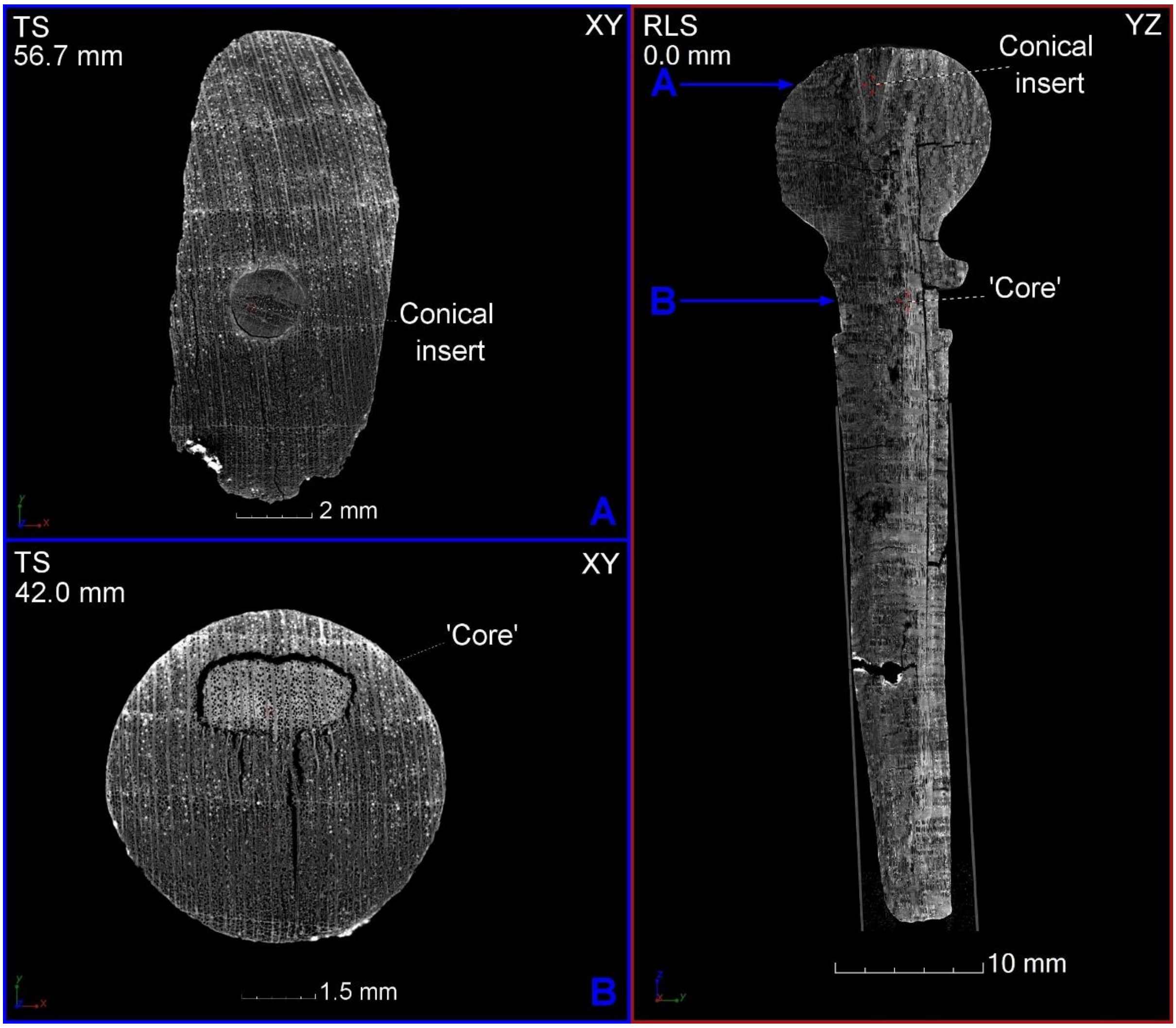
Figure 6. Post conservation (Versa) pipe stopper Stage 2: At 700nm voxel resolution, the bars of the scalariform perforation plates are clearly resolved. Here, the volume has been re-orientated to view the plate face-on (left) as a planar section (PS) indicated by the yellow plane location in the TLS side-on view.



4.2.2 Tuning peg

The conical insert (Figure 7) was made from wood – a gymnosperm: softwood, conifer – which differed from the main peg (an angiosperm: hardwood).

Figure 7. Post conservation (HMX) tuning peg overview scan: Slices showing the location in the RLS (right) of the decorative conical insert A (viewed in the TS plane, left) and inner 'core' B (viewed in the TS plane, left). Note the semi ring porous vessel distribution in the TS.



The µ-CT scanning showed that the body of the tuning peg has an inner ‘core’ of better-preserved wood aligned longitudinally through it. For the most part, this ‘core’ is separate (i.e. internally detached) along its length, and is only attached at the top and bottom of the object (Figure 12). This ‘core’ was used for the wood identification of the peg body. It was identified as a *Prunus* sp. (the cherries genus) with: i) semi-ring porous vessel pattern (Figure 7), ii) noded rays at the ring boundary, iii) rays up to 5 cells wide and up to 80 cells (*c* 1.5mm) high (Figure 8), and iv) the presence of distinct spiral thickenings (Figure 9). Anatomically, the *Prunus* species are very similar and so resolving to species (which is possible in some cases) has been inhibited here by not being able to achieve sufficient spatial resolution. Unfortunately, in spite of two attempts, scan Stage 2 (0.7 µm voxel resolution) was unsuccessful due to this object’s instability. Despite this, it was possible to confidently discount *P. avium* (wild cherry), as the ray heights of that species are too short (Schweingruber 1990, p. 138). Based on ray cell heights observed here (up to *c*. 80), a *P. spinosa* (blackthorn) type is possible, which is a group that also includes *P. cerasifera* (cherry plum), *P. domestica* (wild plum), *P. insititia* [= *P. domestica* ssp. *institia*] (damson) and *P. persica* (peach) (see Schoch *et al* 2004; http://www.woodanatomy.ch/species.php?code=PNPE). The observation in the sample material of some degree of ray cell heterogeneity (sheath cells were observed in places (Figure 8)) would also fit with this group.

Figure 8. Post conservation (Versa) tuning peg Stage 3: TLS slice through the 'core' region at 1 µm voxel resolution showing the multiseriate rays (up to 5 cells wide) and occasional ray sheath cells indicated by the arrow.

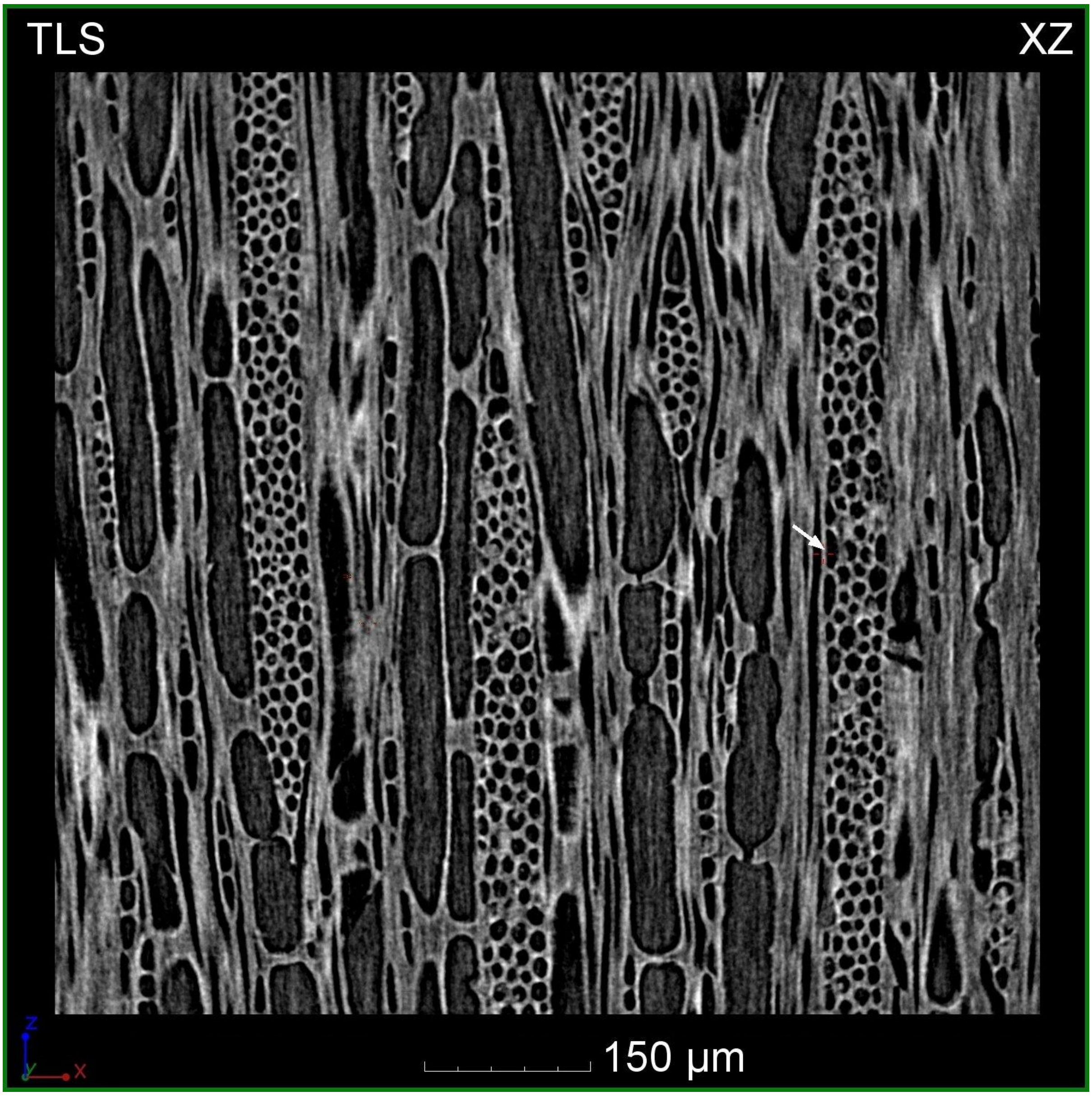
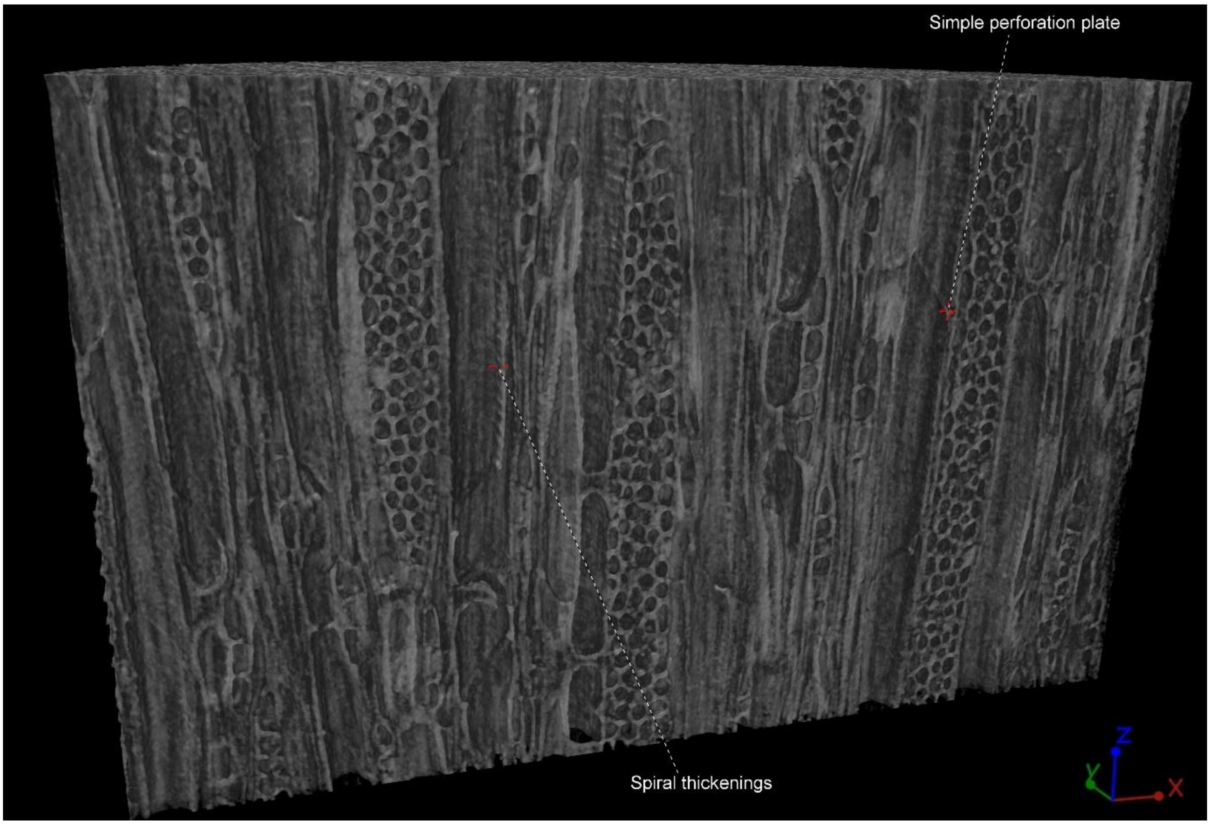


Figure 9. Volume rendering (3D model) of the tuning peg 'core' showing the vessels' spiral thickenings and simple perforation plates.



The tuning peg's cone insert coniferous identification was refined to *Pinus sylvestris* (Scots pine) group with: i) sharp early-latewood boundary (Figure 10), ii) the presence of both axial (Figure 10) and radial resin canals (Figure 11), iii) uniseriate rays (here, typically ≤10 cells high, although a few were >10) (Figure 11), iv) bordered pits (uniseriate alignment) in the axial tracheids (Figure 11), v) absence of spiral thickenings, vi) window pits present in the ray cell walls (Figure 11), and vii) the presence of dentate (tooth-shaped) features in the walls of the ray tracheids (Figure 11). As well as *P. sylvestris* this (wood anatomical) group of taxa also includes *Pinus mugo* (Dwarf mountain-pine) and *Pinus nigra* [consisting of two subspecies, see Stace 2010] – none of which can be distinguished solely on the basis of their wood anatomical characteristics (Schoch *et al* 2004, Schweingruber 1990)[1].

Figure 10. Post conservation (Versa) tuning peg Stage 1: TS slice image through the softwood conical insert showing the abrupt early- to late-wood transition and axial resin canal(s).

[1] Rol (1932) [in Phillips 1941, p. 294] defines the Sylvestris group (7) as including: *P. sylvestris,* and also *P. densiflora, P. nigra* and *P. resinosa*.

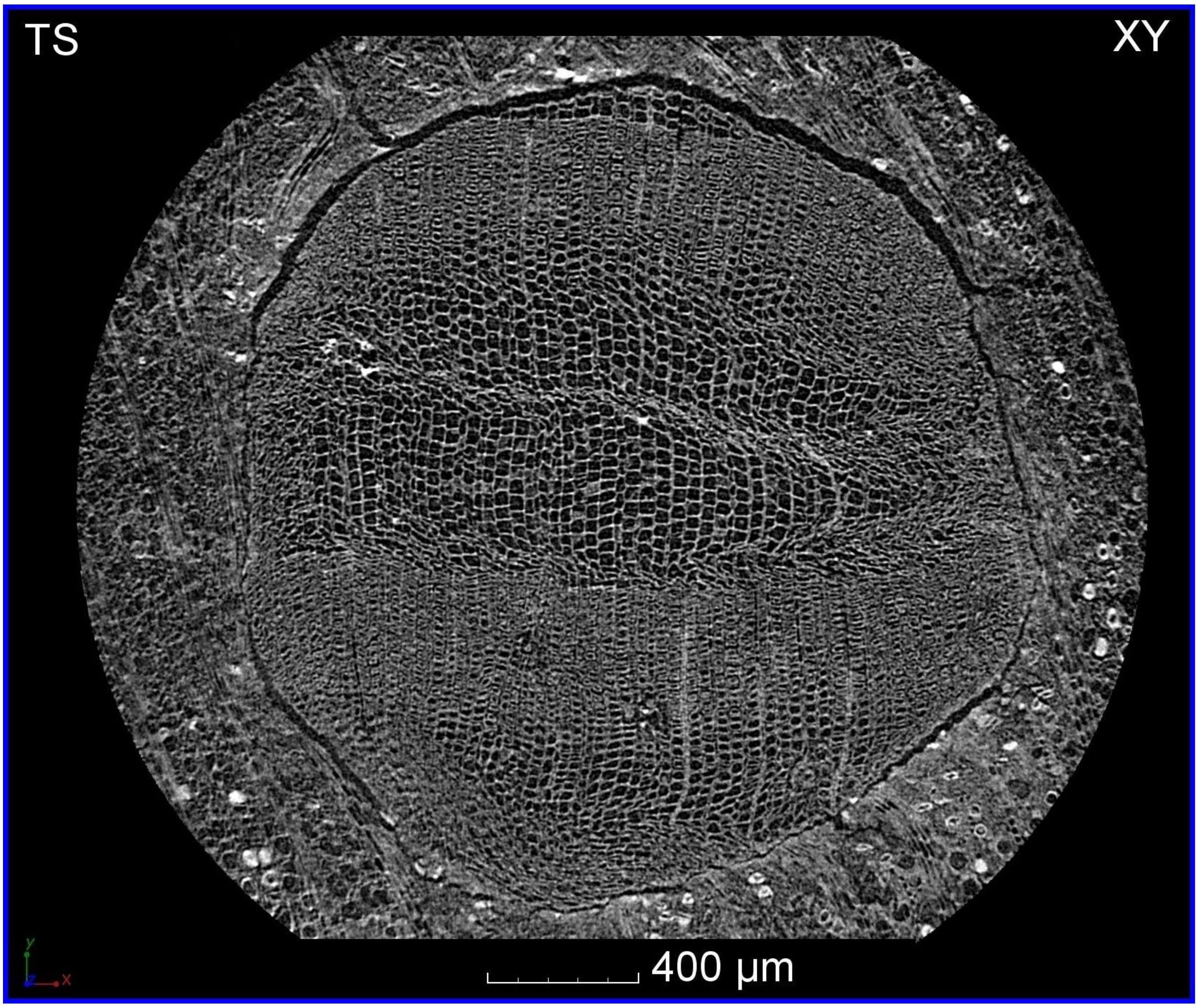
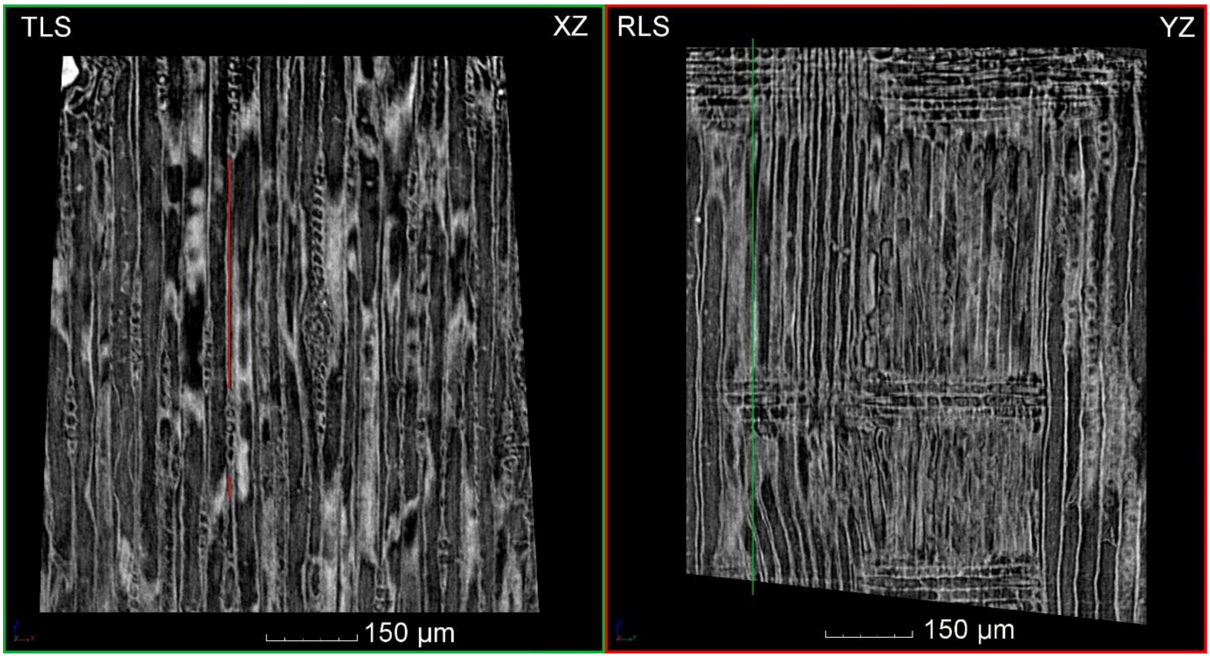


Figure 11. Post conservation (Versa) tuning peg Stage 3: slice images through the softwood conical insert showing uniseriate rays (TLS), an axial resin canal (TLS), single columns of bordered pits in the axial tracheids (RLS), window pits in the ray cells (RLS) and dentate walls of the ray tracheids (RLS). The planar location of the TLS slice (green border, right) is indicated by the corresponding colour line in the RLS (red border, left) slice.



At least three separate growth rings were present, of which the third (i.e. youngest) ring was only very partial (radially). µ-CT also showed the poor condition of the insert, with radially distorted structure, evident as undulations in radially-aligned features that would originally have been linear, e.g. the rays. Whether this is due to the piece being compressed during manufacture, post-depositional decay, the conservation process itself, or a combination, is unclear.

4.3 Other general observations

As well as visualising the wood anatomical structure, µ-CT has also provided insights into the internal structure and condition of the objects. Scans showed a ‘core’ in the tuning peg body in better condition than the surrounding wood, without cracks and splits (Figure 12). The resulting clarity of the anatomical features enabled the secure wood identification.

Figure 12. Post conservation (HMX) tuning peg overview scan: TLS slice image showing the internal cracks and the inner ‘core’. The blue lines indicate the points where the ‘core’ attaches (as determined in the TS plane).

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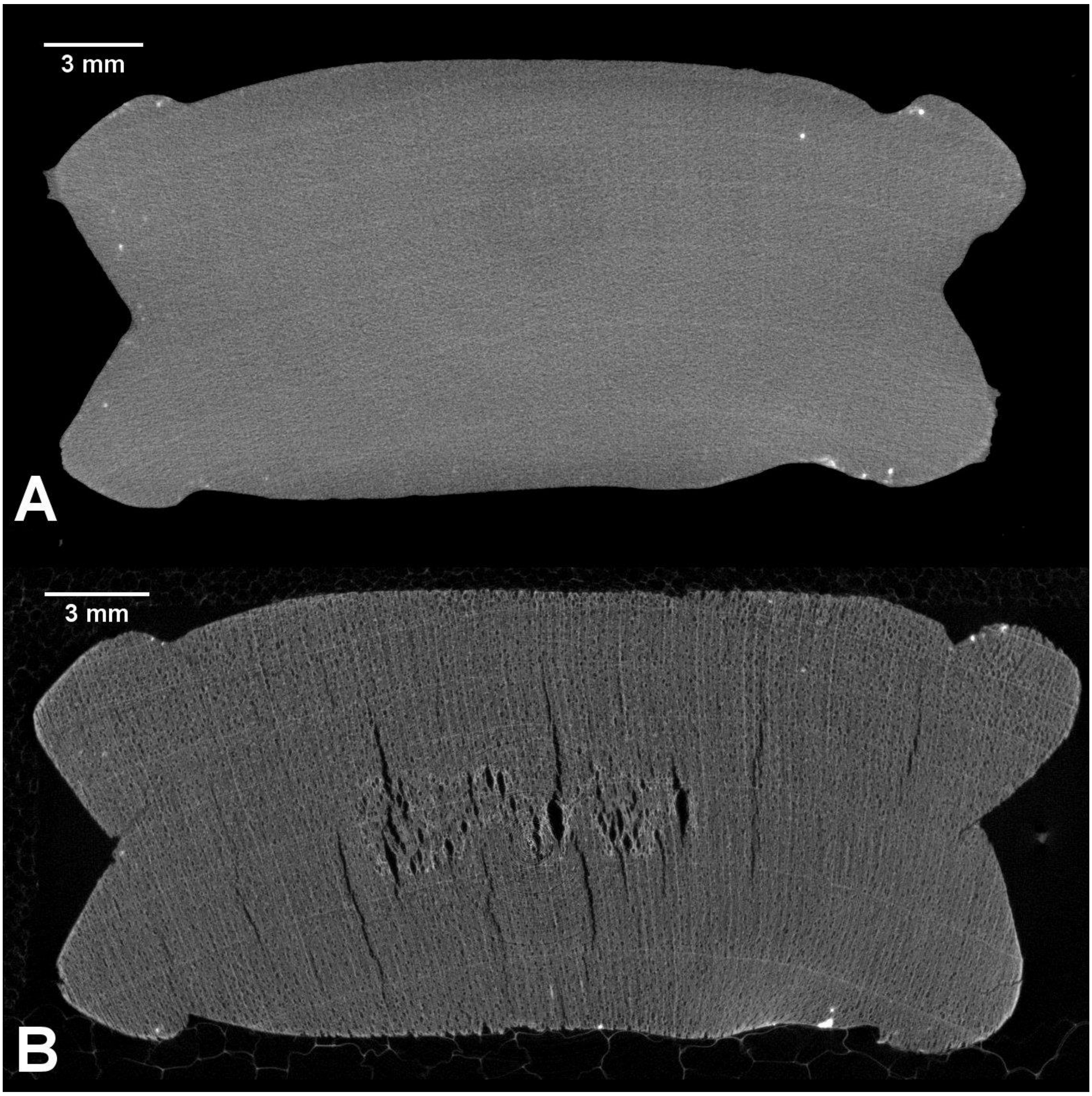
There is a horizontal crack through the peg at the point of the string hole and multiple splits throughout the height (visible in Figures 7 and 12). These are lines of weakness that will be vulnerable to breakage. Internal cracks are visible within the pipe stopper too, manifesting as several radially aligned voids in the centre of its head (Figure 13). It cannot be established whether these were present before conservation due to the low CNR in the waterlogged object pre-conservation µ-CT data. It is possible that PEG 400, which was used during the first stage of impregnation, did not fully freeze and some air-drying took place in the very centre of the pipe stopper resulting in cracks.

Figure 13. Pre- (A) versus post-conservation (B) (HMX) pipe stopper head overview scan slices in the TS plane, show the limited contrast due to water content and internal cracks that became visible post-conservation.



4.4 Implications for future studies of waterlogged wooden objects

Based on these results, if a wooden object is a candidate for conservation (using PEG and freeze-drying) and destructive sampling is not desirable, there seems to be little value in µ-CT scanning before conservation, especially as this study shows that wood identifications are achievable on conserved material. As well as achieving high contrast and resolution, scanning post-conservation also limits CT motion artefacts in the reconstructed volumes (as water in objects can cause movement during the scan) and lifts any restrictions on scan time due to concerns about wet objects drying out.

µ-CT pre-conservation achieved limited image contrast in comparison to µ-CT post-conservation, which made it difficult to resolve some features. Image contrast is governed by variation in X-ray attenuation throughout the specimen. Figure 13 demonstrates how the contrast improves from a wet to a dry µ-CT scan (all that differed in scanning set-up was the voltage –100 versus 80 kVp). On the 'overview' resolution scale, image contrast was more significant for identification of anatomical features when comparing between pre-conservation and post-conservation µ-CT. The Versa enabled phase-contrast edge enhancement from the fringe patterns displayed at boundaries, which helped improve image fidelity and definition of anatomical features in the vessel walls, such as perforation plates. The potential of true phase-contrast lab-based microtomography of wood microstructure was demonstrated by Mayo *et al* (2010), who also commented that weakly absorbing thin-wall structures such as cell walls are more easily resolved. This could not be trialled on the waterlogged objects due to scan time criteria to limit drying.

Post-conservation it was possible to achieve sub-micron resolution, and resolve some of the finest anatomical features, including scalariform bars in the *Betula* sp.'s perforation plates (typically ~3 µm thick bars with ~10 µm spacing) and the *Prunus* sp.'s spiral thickenings on the vessel walls (~2 µm). However, it should be minded that the latter images were produced from the better-preserved 'core' of the tuning peg. Even the highest resolution µ-CT (here 700 nm) could not fully resolve the tiny individual pits in the vessels in the *Betula* sp.; the size criterion for minute pits is ≤4 µm (IAWA 1989, p. 250). When scanning at such high resolutions even the slightest movements would cause misalignment of projection images, resulting in CT motion artefacts in the reconstructed volumes, and necessitating a rigid specimen mount. A stable mounting system was required for both scan-scenarios: before and after conservation. However, there are limitations when mounting/stabilising very delicate objects, as in the case of the tuning peg.

For this work, only two objects and three different wood types were available for study and critical evaluation of µ-CT scanning as a technique for wood identification. The results are promising and it is hoped that over time, wooden objects in collections (which have been conserved in a similar way) that so far could not be identified due to sampling restrictions, will be considered for wood identification to add to our understanding of the use of wood as a resource to manufacture objects.

4.5 Wood use and selection

Three wood types have been securely identified to genus level: *Betula* sp. (birch) (pipe stopper), and *Prunus* sp. (cherries) and *Pinus sylvestris* (Scots pine) group (tuning peg). It is not appropriate to make detailed regionally-based inferences about likely species because the material was recovered from the wreck of an internationally travelled ship, although all the types have species that are native to the British Isles (see Stace 2010). Despite these reservations, some suggestion of possible wood source could be inferred when considered together with other evidence recovered from the ship; for example, the clay tobacco pipes themselves were typical of the London/southeast England region based on bowl typology of the mid-17th century (Higgins 2016), i.e. they were locally-produced and derived.

Prunus sp. "is strong, hard and has a close grain, and is excellent for turnery and carving" (Gale and Cutler 2000, p. 196), so its strength makes it well-suited for taking the strain of the instrument's tightened string. In comparison, *Pinus sylvestris* (Scots pine) is relatively soft and easy to work, so more suitable for the decorative conical insert which was fitted and tightly-set within the peg's body. Pine is pale-coloured, and therefore has the desired effect of being a striking visual comparison with the darker, red-brown coloured surrounding wood used for the body of the peg itself. As well as the wood types used, µ-CT has provided some insight on the construction of the objects; the tuning peg's conical decorative insert, and the alignments of the wood grain. Although both objects have sections (the heads) where their width is greater than the depth, the wood alignments on these parts are different; the pipe stopper has the growth ring alignments parallel to the longest edge, and the tuning peg has the growth rings perpendicular to the longest edge.

5 Summary and conclusions

µ-CT has resulted in the better understanding of the internal structure of these wooden objects through i) the identification of wood type, ii) providing information on the manufacture techniques of the objects (in particular here, the alignment of wood grain, and use of a decorative component), and iii) identifying internal fractures and lines of weakness, vital for appropriate storage, handling and display. These data can be used to inform animations and museum displays, and create replica objects, for public engagement and education purposes.

Based on this study, the primary methodological recommendation is that, for wood identification purposes, µ-CT scanning is carried out on conserved wooden objects (i.e. not waterlogged remains). Ideally, scanning should be carried out at a range of voxel resolutions to assess anatomical features that range on multiple length scales e.g. growth rings (mm to cm), ray heights (µm to mm) and pits in the vessel wall (µm). Although the size of diagnostic features can vary between wood types (e.g.

the size of vessel pits), for the wood types encountered here 1 µm voxel resolution was sufficient to resolve the majority of anatomical features required for identification. 1 µm voxel resolution is advantageous from a scanning perspective as it enables the Versa 510's 4X objective to be used instead of the 20X; this improves the image intensity for a given exposure time so improves SNR or allows the exposure time, and thus scan time, to be reduced.

µ-CT scanning requires specialist equipment and staff and can also be time consuming. The time [cost] available for undertaking the scans and manipulating the image data (as well as liaising with the wood and conservation specialists), needs to be weighed up against the 'knowledge gain' of additional information, and the importance and level of its contribution to understanding and valuing the objects. Table 3 sets out the cost/benefit considerations of this study. If destructive sampling for wood identification is permitted, then that will always be far more cost-effective.

Table 3. Comparison of lab µ-CT and conventional techniques for the purpose of wood identifications, specific to this study.

µ-CT scanning	**Conventional technique**
Non-destructive	Destructive
3D internal inspection – arbitrary re-orientation to identify features of interest	2D surface slice – integrity reliant on wood condition
Large number of slices in one scan	Multiple sampling required
Isotropic sub-micron resolution is achievable with suitable specimen size	Optical magnification up to 400X
1 day	20 minutes
Very large digital archive (GB–TB)	Smaller digital archive [from microscope images] (kB–MB)

The large amount of born-digital data produced through µ-CT studies can be immense, and as such requires adequate storage facilities for its security and longevity, in order to ensure compliance with the FAIR (Findable-Accessible-Interoperable-Reuseable) data principles (Wilkinson *et al* 2016). This will have further cost implications.

This work has successfully demonstrated the role and applications of µ-CT scanning complete, conserved (previously waterlogged) wooden objects, and how best to use the technique, when destructive sampling and/or other imaging techniques (e.g. Synchrotron) are not appropriate/accessible. It shows that the impregnation of PEG together with freeze-drying do not obscure the anatomical features of wood up to the resolutions achieved in this study (for these species), and that successful wood identifications are possible on wooden material conserved in this way.

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7 Acknowledgements

KER and MNM undertook the µ-CT scanning, with KER carrying out the image processing, ZJH carried out the wood identifications and recording, and AMM recorded and conserved the archaeological artefacts. The photographs of the conserved objects were taken by James Davies (Lead Photographer) and Steven Baker (Photographer) (both Historic England).

Many thanks to the following organisations and individuals:

- the wreck’s licensee Steve Ellis, together with Carol Ellis, Steve Meddle and the licensee team
- Historic England for funding project Pr6901 and supporting this additional research
- Cotswold Archaeology for managing project Pr6901
- MSDS Marine who provided diving support for the excavation
- Dan Pascoe, the site nominated archaeologist (2014-2016)
- the µ-VIS X-Ray Imaging Centre, University of Southampton, for allocating the staff and resources to carry out this study, supported by EPSRC grant EP-H01506X. Special thanks to Richard Boardman.

The project archive will be deposited with the ADS and Southend Museum Services. The pipe stopper is currently loaned to, and on display at, the National Maritime Museum, Greenwich, London. Supporting CT data are openly available from the University of Southampton repository at https://doi.org/10.5258/SOTON/D1940. Contact the corresponding author for raw imaging data.

https://historicengland.org.uk/advice/heritage-at-risk/search-register/list-entry/24507

#LondonWreck1665

Finally, many thanks to the anonymous reviewers for their constructive feedback and recommendations.

8 Appendix

Figure A1. The object (here, shaft of the pipe stopper) was mounted for µ-CT scanning with two stage magnification within the Versa to achieve sub-micron resolution.

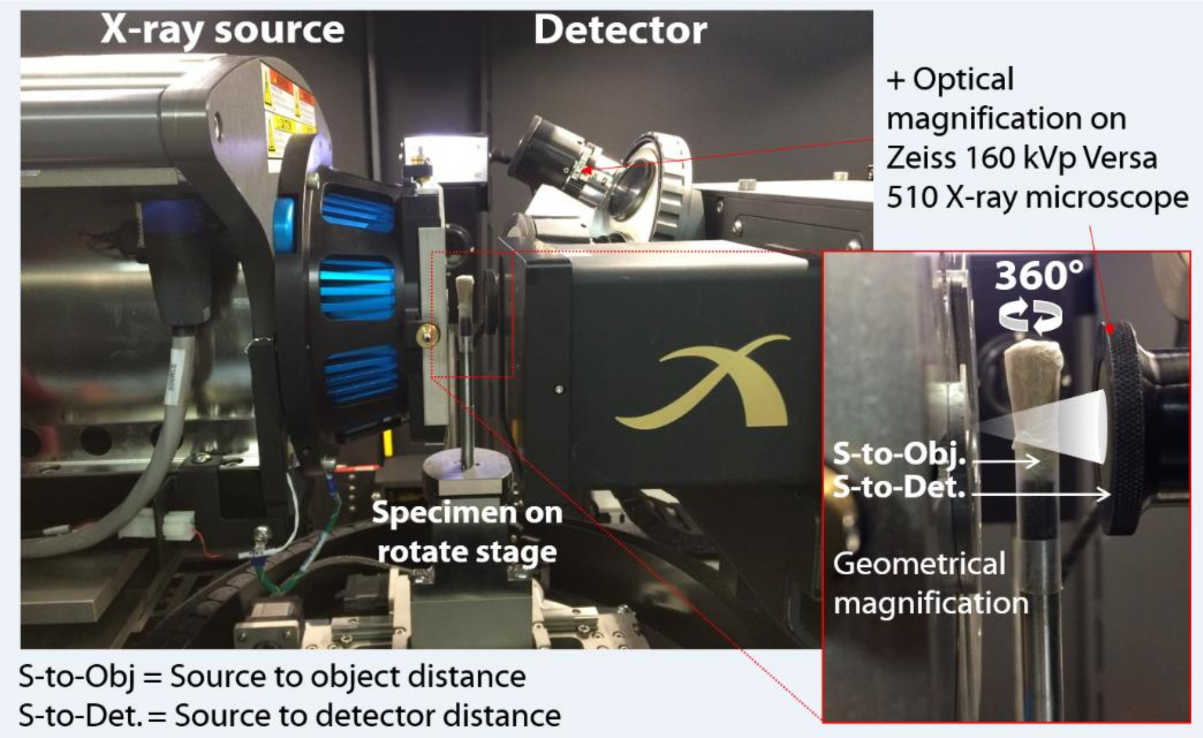


Figure A2. µ-CT volume rendering (3D model) of the pipe stopper head, before conservation (HMX).

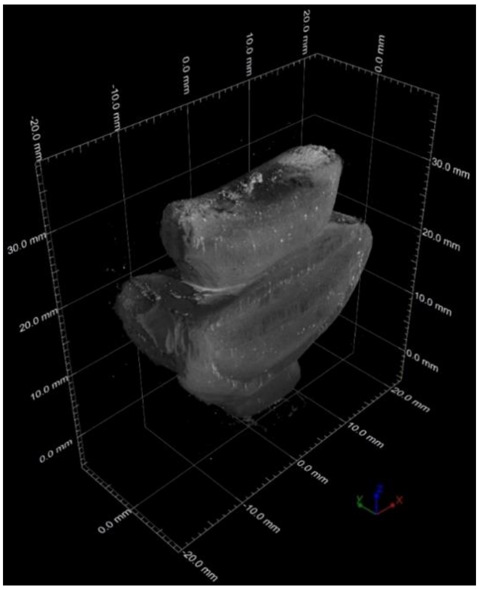
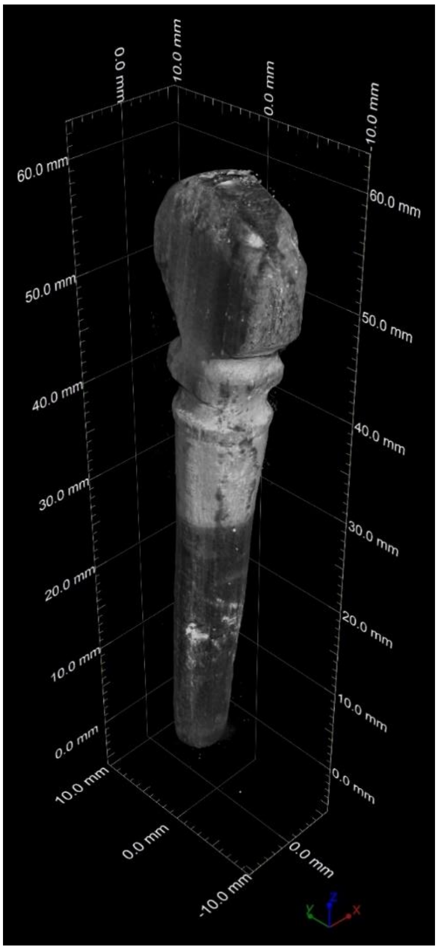


Figure A3. µ-CT volume rendering (3D model) of the tuning peg, before conservation (HMX).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