SHORT REPORT

Possible Radiation-Induced Damage to the Molecular Structure of Wooden Artifacts Due to Micro-Computed Tomography, Handheld X-Ray Fluorescence, and X-Ray Photoelectron Spectroscopic Techniques

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This study was undertaken to ascertain whether radiation produced by X-ray photoelectron spectroscopy (XPS), micro-computed tomography (μCT) and/or portable handheld X-ray fluorescence (XRF) equipment might damage wood artifacts during analysis. Changes at the molecular level were monitored by Fourier transform infrared (FTIR) analysis. No significant changes in FTIR spectra were observed as a result of μCT or handheld XRF analysis. No substantial changes in the collected FTIR spectra were observed when XPS analytical times on the order of minutes were used. However, XPS analysis collected over tens of hours did produce significant changes in the FTIR spectra.

Introduction

Prayer beads are sixteenth century devotional objects constructed of dense wood whose shapes are variable but generally consist of two segments attached by a hinge with the outer surface decorated in complex patterns and an interior showing elaborate, biblical scenes (**Figure 1**). The largest beads are approximately 6 cm in diameter with a depth of approximately 3 cm. The construction techniques used for these important cultural heritage artifacts have been investigated using synchrotron-based X-ray tomography (Reischig et al., 2009) and, more recently, micro-X-ray computed tomography (μCT) was carried out at Sustainable Archaeology, The University of Western Ontario (London, Ontario) on a collection of prayer beads from the Thomson Collection at the Art Gallery of Ontario (Ellis et al., 2012). While X-ray analysis of cultural heritage objects is now widely used (Bergmann, 2007; Dik et al., 2008; Garside and O'Connor, 2007; Janssens et al., 2000; Janssens et al., 2010; Mantler and Klikovits, 2004), surprisingly little work has been undertaken on possible radiation damage to artifacts during analysis

(Bergmann et al., 2012; Grubb, 1974; Hoffmeyer and Hanna, 1989; Richards et al., 2012). Furthermore, Bergmann et al. (2012, 365) specifically state that "X-ray techniques are generally non-destructive at sufficiently low doses. However, X-rays may cause some damage . . . [and] therefore, damage studies must be performed in some cases in order to optimize analysis to the smallest necessary dose".

Barker et al. (1999) and Pandey et al. (1999) have used Fourier transform infrared spectroscopy (FTIR) to identify differences between the lignin and cellulose content of hardwoods and softwoods providing detailed information regarding the molecular structure of wood. In addition, FTIR has been used to examine damage to wood and wood products as a result of fungal attack and ionizing radiation, as well as visible and UV light, the latter usually for long exposures. Accordingly, FTIR was used in the research reported here to ascertain whether or not radiation exposure during analysis using X-ray photoelectron spectroscopy (XPS), X-ray tomography and/or handheld X-ray fluorescence (XRF) equipment resulted in changes to the wood at the molecular level.

The research was conducted in two distinct parts. In the first portion, FTIR was used to monitor changes in the surface chemistry of wood samples as a result of routine analysis using X-ray tomography and handheld XRF. These X-ray techniques were chosen because they are widely used in the examination of cultural heritage artifacts and the exposure times selected are representative of those used in conservation studies. In the second

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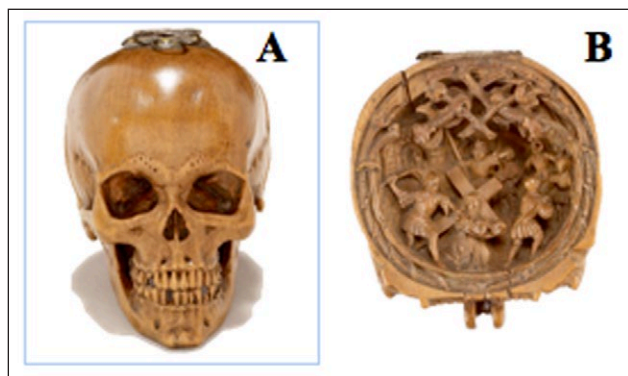Corresponding author: Madalena Kozachuk (mkozachu@uwo.ca)

Figure 1: Prayer bead, boxwood, AGO ID.29283. **(A)** Exterior **(B)** Interior carving showing the Carring of the Cross. The Thomson Collection of European Art © Art Gallery of Ontario.

portion, FTIR was used to monitor the effect of large X-ray doses imposed via XPS to wood samples in order to deliberately induce changes to the wood chemistry such as the loss of functional groups (i.e., ethers and alcohols), which would lead to water loss and crosslinking, ultimately leading to darkening/charring of the surface and changes in mechanical strength. This approach provides a concrete comparison of a purposefully altered sample, indicative of long-term damage to the samples analyzed by typical X-ray sources in conservation studies. This was done to help establish safe time limits and energy levels for X-ray exposure. Consequently, the presented results are relevant to contemporary researchers.

Methods and Materials
Wood sample
A piece of San Domingo Boxwood, approximately 8 × 2.5 × 2.5 cm, was supplied by the Art Gallery of Ontario. This sample was identified by Arlen Heginbotham of The J. Paul Getty Museum as *Phyllostylon rhamnoides*. While the Thomson Collection prayer beads are made from a different species of boxwood (believed to be European Boxwood, *Buxus sempervirens*) this sample has similar density and workability, both traits ideal for carving, and was used to serve as a surrogate for the wood used in the construction of the artifacts. In addition, the results obtained might be expected to apply to any dense wood hard enough to allow for fine carving. Individual subsamples for each subsequent treatment were cut from this piece using a steel chisel or a surgical blade as appropriate to the size requirement of the analytical method(s) of choice.

X-ray photoelectron spectroscopy
A subsample of wood for analysis by X-ray photoelectron spectroscopy (XPS) was cut from the parent sample using a sterile stainless steel scalpel to obtain a piece of approximately 10 × 5 × 2 mm. The sample was then mounted on a carbon stud prior to analysis. XPS analysis was carried out at Surface Science Western, University of Western Ontario, using a Kratos AXIS Ultra XPS and a monochromatic Al K$_\alpha$ X-ray source with a current of 15 mA and an energy of 14 kV (210 W) producing an X-ray analytical area of 300 × 700 μm at 1.5 – 2 × 10^{-9} torr base vacuum. The

sample was subjected to two analysis times: one of 44 hours throughout which photoelectron spectra were collected every 25 minutes and a second of 13 hours with spectra collected every four minutes. High-resolution carbon *1s* spectra were obtained in order to monitor changes in the chemistry of organic species. The longer radiation exposure and resulting high X-ray dose was used to identify functional groups sensitive to X-ray exposure, while the shorter analysis, having only 4 minutes between spectra, could be used as an indicator of early changes in the sample.

Micro-computed tomography
A subsample of wood measuring approximately 2.5 × 2.5 × 1 cm, removed from the parent sample using a stainless steel scalpel, was subjected to micro-computed tomography (μCT) at Sustainable Archaeology using a Nikon Metrology XT-225 industrial μCT scanner with a 3 μm spot size from a tungsten reflecting target. The instrument voltage was 105 kVp at a current of 48 μA, with a 500 μs exposure and 2 frames averaged per projection. The total scan time was 53 minutes.

Handheld X-ray fluorescence spectroscopy
Two subsamples, approximately 1 × 1 × 0.5 cm, used for analysis with the handheld XRF instrument, were removed from the parent sample using a stainless steel chisel in the University of Western Ontario physics machine shop. The analytical instrument used was a Bruker TRACER-IV (Shugar & Mass, 2012) that produced an oval beam 0.5 cm at its widest point when projected on the sample surface. Two pieces of boxwood were examined using a 40 keV X-ray at an anode current of 13 μA. Two locations on one piece were exposed for 60 seconds and 3 locations were exposed for 120 seconds on the other. In all cases, locations were selected such that there was no overlap between sites. The exposure times were taken to be typical of those used in routine field analysis.

Fourier transform infrared spectroscopy
All FTIR spectra were obtained using a surface reflectance, Bruker IFS 55 (SR-FTIR) spectrometer at Surface Science Western. The instrument was equipped with an IRScope microscope with an attenuated total reflection (ATR) objective equipped with a germanium crystal. The ATR objective probes an elliptical area approximately 80 × 100 μm having an estimated collection depth of 1–2 μm and a resolution of 4 cm^{-1}. There were 100 background scans collected from the air and these were used to generate an average background spectrum, which was subtracted from subsequent runs. A total of 10 FTIR spectra were obtained from the sample that was analyzed by micro-CT and a total of 9 and 7 spectra were obtained from the samples exposed under XRF for 60 or 120 seconds, respectively.

A reference spectrum was prepared in the following way using an unexposed subsample of the boxwood. FTIR spectra were obtained at 30 sites chosen at random from the sample surface with a rotation of 90° after 15 spectra were collected to compensate for the heterogeneity of the surface. A three-point baseline correction was performed

on each spectrum using straight lines drawn between wavenumbers 3680, 1850 and 825 cm⁻¹. These 30 spectra were then averaged to produce a reference spectrum for all subsequent analyses. The functional groups used to identify possible damage to the wood by comparison with the reference spectrum were selected as they were characteristic of lignin and cellulose components found in each spectrum (Barker & Owen, 1999; Ferraz et al., 2000; Garside & Wyeth, 2003; Pandey, 1999; Pandey & Pitman, 2003) (**Table 1**). Additionally, they are representative of the structural units that were visibly charred during the XPS analysis. Alkyl $C – OH$ and $C – O – C$ from cellulose, whose absorbance is observed at 1033 cm⁻¹, was chosen to monitor any X-ray induced chemical variation. The absorbance at 3347 cm⁻¹ is associated with the $O – H$ vibrational mode in water and is sensitive to relative humidity and the ultra-high vacuum in the XPS instrument. This absorbance is not included in any further analysis. All absorbance positions are compared using absolute peak height.

Statistical analysis

Since all analytical samples were derived from the same piece of boxwood, it was impossible to get independent, biological replicates making detailed statistical analysis inappropriate. Nonetheless, multiple measurements were taken from each sample to reflect variable changes to boxwood molecular structures in response to the various X-ray treatments. The following analyses were carried out to obtain an unbiased assessment of whether or not the molecular structure differed between X-ray treated samples and the reference sample. For the μCT data, a Mann-Whitney U test was applied as the data did not meet the assumptions of a parametric t-test; the data were not normally distributed and the variances were not homogeneous. A one-way ANOVA was run on the handheld XRF data to compare the treatments of beam-analyzed samples at 60 and 120 seconds with the reference samples. Both normality and homogeneity of variance were confirmed prior to running the ANOVA. All the above statistical analysis was carried out using SigmaPlot version 11.1. The absorbance at 1033 cm⁻¹, seen in the FTIR spectra following the XPS analyses, was compared to that of the reference sample with a Student's t-test using Excel 2007.

FTIR Absorbance Location (cm⁻¹)	Vibration	Structural Unit
3347	O–H	Cellulose, Lignin, Absorbed H_2O
1596	Aromatic skeletal C–C	Lignin
1508	Aromatic in-plane C–C	Lignin
1369	C–H	Cellulose
1033	Alkyl C–OH	Cellulose

Table 1: FTIR absorbance: assigned vibrations and corresponding structural units (Barker & Owen, 1999; Collier et al., 1992; Ferraz et al., 2000; Garside & Wyeth, 2003; Pandey, 1999).

Results and Discussion

The photoelectron yields associated with selected organic functional groups as a function of time for both 13 and 44-hour XPS exposures are shown in **Figure 2**. These spectra were not normalized since they were run specifically to establish the scale of measurable damage that would occur after long exposure to the X-rays. Neither treatment caused any change in photoelectron yield during at least the first 10 minutes of analysis (this is clearly visible in the spectra obtained during the 13-hour XPS exposure when results were collected every 4 minutes). Longer exposure times induced an increase in photoelectrons characteristic of $C – C$ and $C – H$ functionality with a co-incident decrease in $C – O – C$ and $C – O – H$ consistent with the charring that was visible on the sample surface. $C = O$ and $O – C = O$ show much less variability with time suggesting that these groups are more stable under X-ray exposure.

After the 44-hour exposure to XPS X-rays, the FTIR analysis (**Figure 3**) showed changes near 1033 cm⁻¹ associated with alkyl $O – H$ and $C – O – C$ bonds. A Student's t-test confirmed a change at 1033 cm⁻¹ in the FTIR spectrum as a result of the X-ray exposure.

The FTIR data from the μCT exposed wood were examined via a Mann-Whitney U test (**Figure 4A**) and the FTIR data from the handheld XRF exposed wood were examined using a one-way ANOVA (**Figure 4B**). The analysis performed on the μCT data revealed no difference between the scanned surface and the reference sample. Furthermore, the FTIR spectra of the wood surfaces exposed to the handheld XRF for 60 or 120 seconds did not differ from each other or from the reference sample.

Statistical analyses were done simply to obtain an unbiased assessment of whether or not the molecular structure differed between X-ray treated samples and the reference sample. These statistics were collected from the same parent block and therefore can only provide us with the effect of X-rays on this single piece of San Domingo boxwood. However, the changes induced in this wood sample should serve to caution conservators to limit the time of exposure to X-ray sources when studying dense, wooden artifacts, such as prayer beads. However, short exposure during analysis using the XPS, μCT or XRF equipment used here did not produce any significant changes to the structure of the wood after analysis times typical of routine investigations.

The XPS treatment was deliberately undertaken to expose the wood surface to a highly localized X-ray dose. There was some visible charring and the resulting FTIR spectrum differed from that of the reference sample; specifically, a marked change occurred at approximately 1033 cm⁻¹ as a result of XPS analysis. This absorbance corresponds to alkyl $O – H$ and $C – O – C$ ether linkages. Extensive damage will lead to loss of water and carbonate ions. Prior to this, changes such as carbon chain free radial induced cross-linking, leading to changes in mechanical strength, might be anticipated (Bouchard et al., 2006; Colom et al., 2003). The photoelectron spectrum from the 44-hour analysis (**Figure 2A**) showed an increase in the $C – C$ and $C – H$ peaks consistent with the observed charring along with an attendant decrease in the electron yield for the $O – H$ and $C – O – C$. The 13-hour analysis (**Figure 2B**)
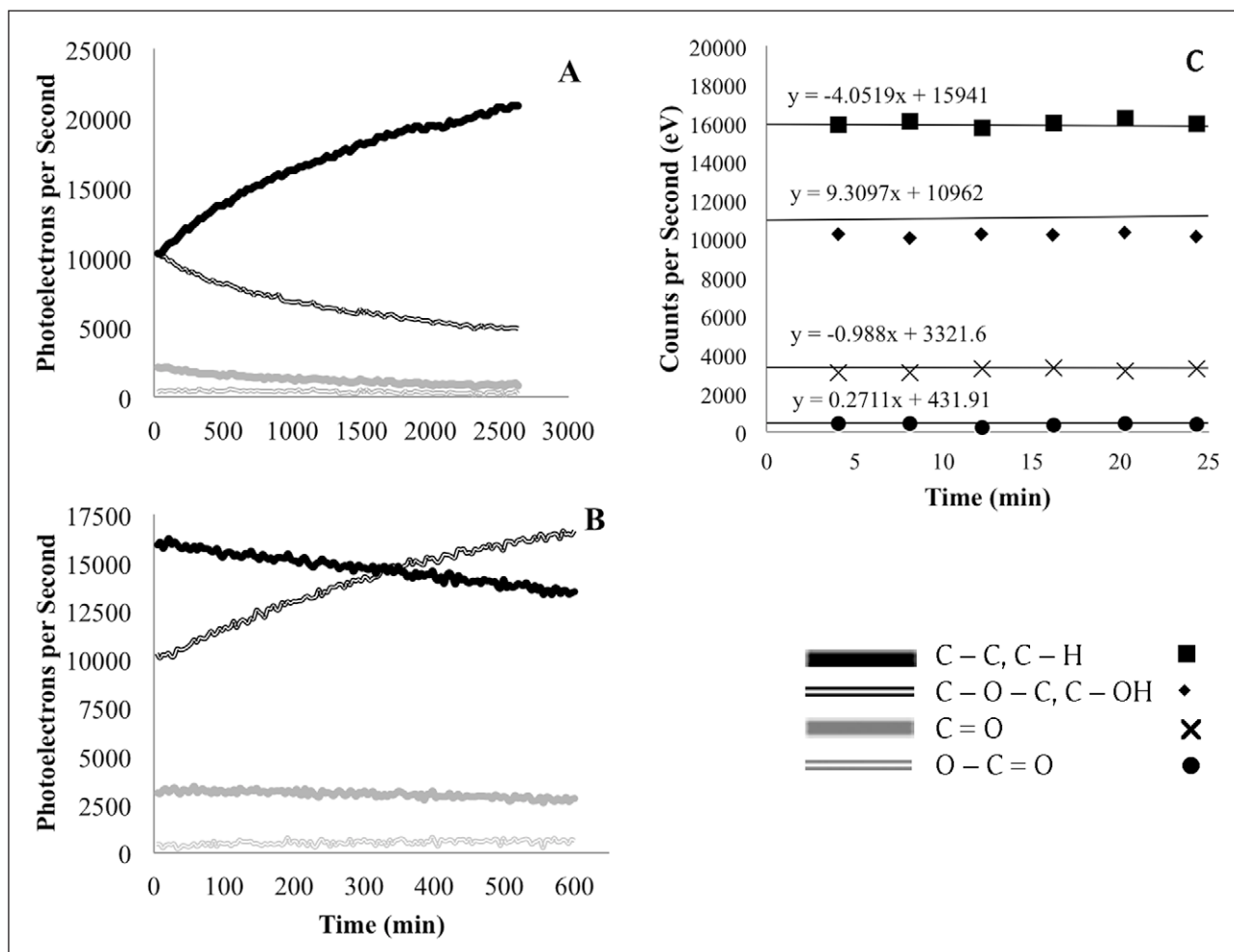
Figure 2: X-ray photoelectron spectra of high-resolution carbon *1s* **(A)** 44-hour experiment with data collected every 25 minutes **(B)** 13-hour experiment with data collected every 4 minutes **(C)** first 25 minutes of the 13-hour experiment.
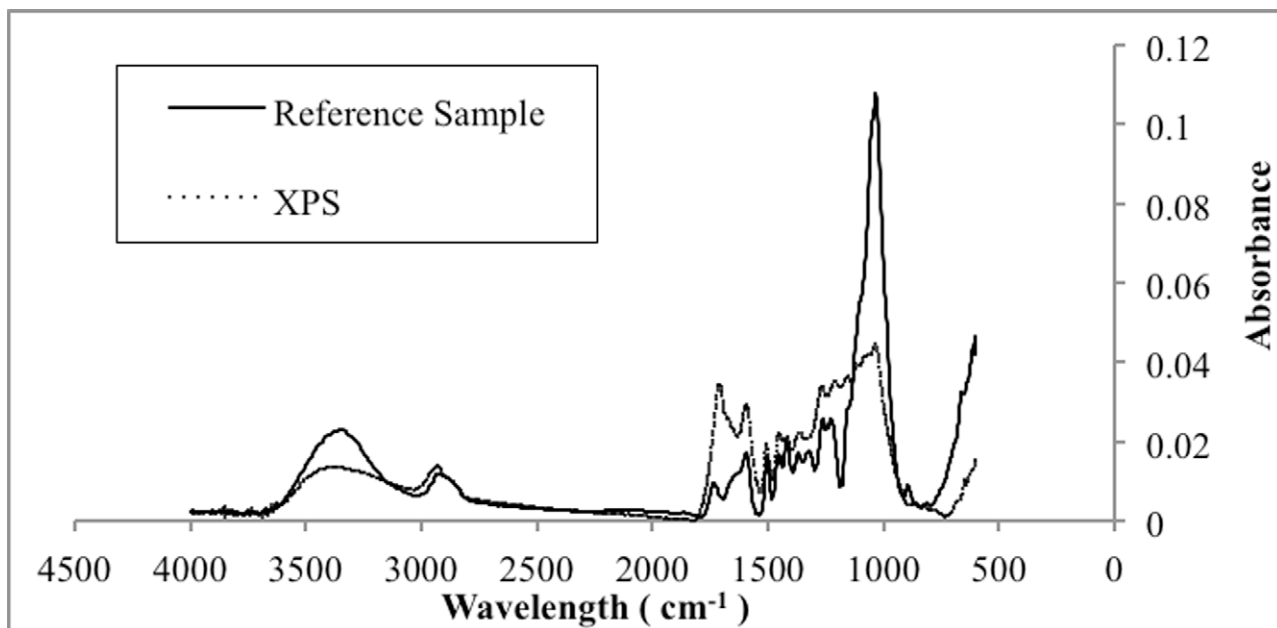

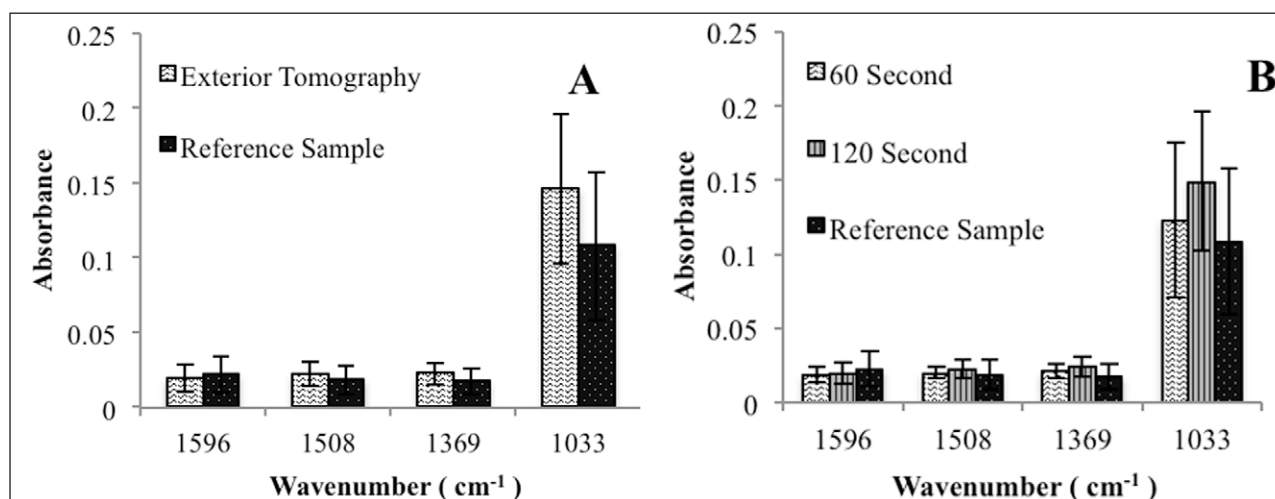**Figure 3:** FTIR spectrum before and after 44-hour exposure to XPS.

Figure 4: FTIR peak absorbance values for boxwood: **(A)** μCT scanned sample compared to reference **(B)** sample exposed to handheld XRF for 60 or 120 seconds compared to reference. For each data set, no differences in absorbance at any measured wavenumber were detected (μCT: $U \le 66.0$, $P > 0.140$; XRF: $F \le 1.436$, $P > 0.159$).

showed a similar result while the better time resolution indicates that no significant change occurred to the sample during, at least, the first 20 minutes of the exposure. It is important to note that X-rays do not interact strongly with elements with low atomic numbers. However, beam energy, current, and exposure time need to be carefully considered prior to artifact analysis to ensure that no surface damage occurs as a result of scientific examination.

The principal value of this result is that it shows that FTIR and XPS are effective techniques for investigating X-ray induced changes in the structure of wood. However, the latter technique, requiring an ultrahigh vacuum, may cause significant dehydration of the wood's surface.

Conclusions

Given the caveats attendant on our statistical analysis, the results show that, under the specific conditions used in this experiment, which sought to replicate typical analytical protocols, there is no apparent damage to wood as a result of μCT or handheld XRF treatments. No change occurred in the XPS spectra for exposure times of less than 10 minutes.

The deliberate attempt to damage the sample using prolonged XPS analysis showed that the alkyl $C - O - H$ and $C - O - C$ bonds are sensitive to X-rays, thus revealing that limiting the exposure time of the sample is one of the precautions necessary to avoid damage when using any form of ionizing radiation – especially with valuable cultural heritage artifacts.

Competing Interests

The authors declare that they have no competing interests.

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