

RESEARCH ARTICLE

# On the impact of micro-CT scanning on radiocarbon dating of fossil material: A cautionary note for the palaeoanthropological community and beyond

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## Abstract

In this study, we investigate the impact of X-rays produced by conventional mCT instruments on fossil materials dated by radiocarbon. Our results clearly show a decrease on the collagen preservation in fossil and modern bones and teeth, and therefore on the radiocarbon analytical results (in particular, the collagen yield and, possibly, stable isotope composition), after mCT scanning. In other words, all the samples analysed here have experienced a noticeable radiation damage, regardless of their nature (bone and dental tissue) and age (modern and fossil). Given these observations, a prudent approach would be for radiocarbon laboratories to expect lower collagen yields for samples that have been previously mCT scanned and ensure appropriately sized standards are processed alongside these samples. Additionally, samples with originally low collagen yields might become unsuitable for radiocarbon dating after mCT or at least show a yield lower than the usual minimum cut-off value. In this case, it might be viable to extend the collagen yield quality assurance parameter for mCT scanned bones and teeth and instead focus on the C:N ratio as the most appropriate indicator of collagen quality, although we cannot exclude that the latter may also be impacted by X-ray exposure. Further investigations on a larger set of samples are required to confirm these first observations. Nevertheless, in the light of these results, we can reasonably conclude by recommending caution regarding the systematic and unlimited use of mCT scanning in palaeoanthropology or in other related disciplines involving fossil material.

## Introduction

In palaeoanthropological research, the study of human fossils is governed by the necessity to cause minimum damage to these rare and valuable remains (e.g., Fox and Hawkes 2019). In this context, micro-computerized tomography (mCT) scanning is now routinely used to record a high resolution three-dimensional reconstruction of samples, enabling to obtain critical information about fossil specimens and extinct species (e.g. García-Campos et al. 2020; Hernaiz-García et al. 2024; Martín-Francés et al. 2018; Smith et al. 2010), and create a digital archive of fossils that may be destructively analysed later. These destructive analyses may be carried out for a wide range of purposes, including palaeoenvironmental or palaeodietary reconstructions, mobility patterns, ancient DNA (aDNA) and geochronology. In particular, a few dating methods may be directly applied to fossil remains, such as radiocarbon, U-series and Electron Spin Resonance (ESR) (e.g., Grün 2006; Grün et al. 2010). Among

them, radiocarbon dating is by far the most widely used for fossils whose chronology is younger than 50 ka (e.g., Grün 2006; Hajdas et al. 2021). It is therefore crucial to establish whether mCT scanning procedures routinely employed in palaeoanthropology have a negative impact on the radiocarbon analysis of collagen samples, and, if any, to determine how this impact can be reduced. This has been very little investigated so far to our knowledge, despite extensive literature showing the ability of ionising radiation to alter the material it interacts with (e.g., Bertrand et al. 2015 and references therein).

Indeed, mCT scanning uses the interaction of X-rays with matter for imaging purpose. While X-rays are by definition ionising radiations that may induce some non-negligible changes in materials at the molecular or atomic level, mCT scanning remains nevertheless widely regarded as a non-destructive technique in palaeoanthropology (e.g. Olejniczak and Grine 2006; Tafforeau and Smith 2008; Tafforeau et al. 2006; or more recently, Mandl et al. 2022). This is because it usually does not cause any major visual damage to the fossils, which is the main criterion typically employed to evaluate the destructiveness of a technique (see Bertrand et al. 2015), although a change of colour (sometimes temporary) in fossils after X-ray synchrotron microtomography has been reported in a few works (Richards et al. 2012; Tafforeau and Smith 2008). Previous investigations have nevertheless showed that X-ray exposure may have a non-negligible ‘radiation-induced side effect’ (using the terminology employed by Bertrand et al. 2015; p. 131) on both the organic and mineral component of modern and fossil bones and teeth (e.g., Bailey 1964; Bowes and Moss 1962; Duval and Martín-Francés 2017; Grieshaber et al. 2008; Grün et al. 2012b; Immel et al. 2016; Rahman et al. 2018; Sauer et al. 2022). The magnitude of this effect is however largely dependent on the radiation dose absorbed by the material, which is directly proportional to the intensity of the irradiation source. This is why it is essential to make a distinction between conventional (*sensu* Immel et al. 2016), or commercial, mCT instruments, which are widely used in palaeoanthropology (and are the main focus of the present work) as part of routine scanning procedures for human fossil remains, and Synchrotron X-ray sources. Comparatively, the latter provide a significantly higher analytical resolution but are less frequently employed within the community. In that regard, Bertrand et al. (2015) point out that, “In the X-ray range, synchrotron beams are orders of magnitude more intense and brighter than conventional laboratory sources” (p. 129).

Only very few studies have examined the impact of X-rays produced by conventional mCT instruments on fossil materials. While X-ray exposure is known to create a radiation-induced ESR signal in tooth enamel that can be used for dating or dosimetric purpose (e.g., Grün et al. 2012a; Yu et al. 2022), the increasing need to directly date human fossils older than 50 ka (Grün and Stringer 2023) triggered a couple of studies specifically centred on mCT scanning. First, Grün et al. (2012b) initially estimated the dose values given to fossil tooth enamel by CT-scanning to be of several hundreds of Grays (Gy). More recently, Duval and Martín-Francés (2017) confirmed the significant impact of mCT scanning on the intensity of the ESR signal of fossil tooth enamel and estimated that conventional instruments are likely to add a few tens of Gy to fossil teeth when using standard experimental conditions. In contrast, when the metallic filter is removed, the X-ray dose absorbed by the sample may exceed 100 Gy, reaching a dose value of the same order of magnitude as in Grün et al. (2012b). The differences between the two studies are likely to be explained by the use of distinct acquisition parameters and mCT instruments. Regardless, these results consistently demonstrate that mCT scanning may lead to significant ESR age overestimations if the additional X-ray dose absorbed by the sample is not taken into account in the calculation.

In another work centred on fossil biomolecules and aDNA preservation in fossil bones, Immel et al. (2016) observed a clear correlation between decreasing aDNA quantities and increasing X-ray dose-levels above 2000 Gy. They also report no significant damage (i.e., nucleotide misincorporations) below 200 Gy, which is typically the range of dose given by conventional mCT scanners when using a metallic filter. However, a closer look at the Figure 2 of Immel et al. (2016) actually shows a significant reduction (~ 30–35%) of the aDNA quantitation after a 200 Gy irradiation, with a very sharp decline with increasing dose, suggesting nevertheless a non-negligible effect induced by mCT scanning at relatively low dose levels. The comparability of the integrated dose stated by this study with the dose measured by ESR in mCT (e.g., Duval and Martín-Francés 2017; Grün et al. 2012b) is difficult to

establish, as Immel et al. quantified the water equivalent surface dose. However, it appears that an increasing dose does have an impact on aDNA quantitation and may lead to significant nucleotide damage when using Synchrotron X-ray sources.

In radiocarbon dating, we are particularly concerned about the potential breakage of the protein component of bone and dentine, which primarily consists of collagen and is knowingly impacted by X-rays (Bailey 1964; Bowes and Moss 1962; Rahman et al. 2018). This protein consists of a triple helix that is insoluble, a characteristic exploited by routine radiocarbon pretreatment protocols where crude collagen is extracted by demineralization in acid (Longin 1971). However, the peptides become soluble once the helix cross-links are disrupted and/or the peptide chains are fragmented, meaning endogenous protein can be lost during demineralization (Marom et al. 2013). Further loss can occur later during during the ultrafiltration step in the pretreatment, where small fragments of the now solubilized gelatin are removed prior to dating (Brock et al. 2013; Brown 1988). Thus, it is possible that radiocarbon dating of bones or teeth that have been exposed to X-rays via a conventional mCT instrument may be more difficult because collagen yields are lower.

In the present work we examine whether mCT scanning using a conventional instrument and a range of standard acquisition parameters may influence radiocarbon analytical results, and especially collagen preservation, obtained from a few modern and fossil bones and teeth.

# 1. Material and methods

## 1.1. Samples

We selected two types of samples: one fossil bone (sample ID: ZAGLIK) from a woolly rhinoceros found at the Marine Isotopic Stage (MIS) 5 locality of Zaglik, Irkusk, Russia, and commonly considered as a laboratory standard reference with multiple radiocarbon dates >50 ka (Wood et al. 2023) at the Australian National University (ANU) Radiocarbon Facility, and one tooth sample (M2) belonging to a modern pig (roadkill) from France (sample ID: FP\_LM2). Several samples were cut from the ZAGLIK bone and the dentine of FP\_LM2: one was directly analysed for radiocarbon (ZAGLIK and FP\_LM2) and the others were submitted to various mCT analyses (labeled A, B, C; see Table 1). Samples were taken from neighbouring areas of the bone/tooth to ensure they were as similar as possible in terms of preservation and minimize the uncertainty related to spatial heterogeneity.

## 1.2. mCT conditions

The samples were mCT scanned with a GE Phoenix v/tome/x\_s 240 instrument at the University of Bordeaux (PACEA/PLACAMAT), France. We selected the set of parameters based on those typically used to scan human fossils (e.g., Martín-Francés et al. 2022; Martín-Torres et al. 2021; Zanolli et al. 2019): 80–180 kV, 100–300  $\mu$ A, a 0.1 mm Cu filter and voxel size < 22  $\mu$ m (Table 1). The mCT instrument and experimental conditions are similar to those previously employed by Duval and Martín-Francés (2017), ensuring thus the acquisition of comparable data.

**Table 1.** Overview of the main acquisition parameters employed during mCT experiments

Sample ID	Type	Number of images	Voltage (kV)	Current ( $\mu$ A)	Filter
FP_LM2_A	Dentine	2550	100	120	0.1 mm Cu
ZAGLIK_A	Bone	2550	80	100	0.1 mm Cu
ZAGLIK_B	Bone	2550	120	200	0.1 mm Cu
ZAGLIK_C	Bone	2550	180	300	0.1 mm Cu

**Table 2.** Effect of mCT scanning on radiocarbon dating

Sample ID	Subsample	mCT scanned	ANU internal lab number N#	S-ANU#	F <sup>14</sup> C ± error	Radiocarbon age (yr BP)
FP_LM	1	No	22890	68118	1.0622 ± 0.0027	Not calculated (modern roadkill)
FP_LM2_A	1	Yes	22891	68119	1.0544 ± 0.0025	Not calculated (modern roadkill)
ZAGLIK_mCTA	1	Yes	21318	65433	0.0010 ± 0.00137	>44900
ZAGLIK_mCTB	1	Yes	21321	65418	0.0024 ± 0.00129	>42600
ZAGLIK_mCTC	1	Yes	21324	65419	0.0004 ± 0.00163	>45100

### 1.3. Collagen extraction

Collagen was extracted using an ultrafiltration protocol at the ANU. After removing the surface with a handheld Dremel drill, bone was crushed using a pestle and mortar to a coarse powder and dentine drilled. Thus, aliquots of the same sample were roughly homogenized before pretreatment.

Pretreatment consisted of reaction with HCl (0.5 M, RT-5°C, overnight), NaOH (0.1M, RT, 30 min) and HCl (0.5 M, RT, 1 hour), followed by gelatinization (0.001M HCl, 70°C, 20 hours), filtration (Ezee™ Filter) and ultrafiltration (Vivaspin™ VS15Turbo 30 MWCO, polyethersulphone membrane) (full details are given in Wood et al. 2023). Cleaned samples were converted to graphite by combustion in a sealed tube with CuO wire and Ag foil, and CO<sub>2</sub> was cryogenically collected and purified before reaction with H<sub>2</sub> over an Fe catalyst. Samples were dated on a NEC SS-AMS (Fallon et al. 2010). Radiocarbon dates were calculated following Stuiver and Polach (1977) using an AMS derived δ<sup>13</sup>C, and a collagen specific background correction was made (Wood et al. 2023). Carbon and nitrogen stable isotope and elemental abundance of the tooth dentine collagen was analysed using an ANCA-GSL connected to a Sercon 20-22 IRMS operating in continuous flow mode using an in-house gelatin reference. Data was scaled using USGS40, USGS65 and IAEA-C6, and data accuracy assessed with IAEA-600 and USGS61 (Wood et al. 2023). All other samples were measured at the Oxford Radiocarbon Accelerator Unit on a similar instrument, but against an internal alanine standard and using USGS40 and USGS41 to scale the data. In both laboratories, uncertainty was assessed by repeat measurement of the internal standard, and was less than 0.1‰, at 1 sigma for both δ<sup>13</sup>C and δ<sup>15</sup>N.

To reduce potential variables, scanned and unscanned samples were of the same starting mass and were pretreated at the same time and by the same person. For samples with sufficient amount of material, several subsamples were analysed and went through pretreatment separately. Collagen yield is calculated as collagen mass (mg) divided by starting mass (mg), multiplied by 100. Numerical results are given in Tables 2 and 3.

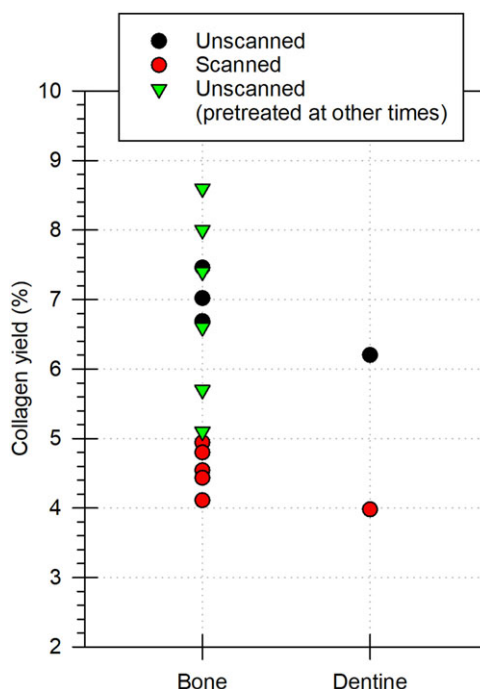
## 2. Results and discussion

Radiocarbon analyses of the scanned ZAGLIK samples return minimum C-14 ages of >42,600 to >45,100 BP, i.e. in agreement with the known MIS 5 age (Table 2). In other words, we do not observe any significant impact of mCT scanning on the radiocarbon age obtained.

In contrast, collagen yields are significantly reduced (Table 3). For unscanned bone samples, collagen yields were around 7.1%. After mCT scanning, collagen yields were reduced to <5%, suggesting protein is damaged during the scanning process. The scanned and unscanned samples clearly cluster in two groups (Figure 1) showing a reduction of 33% to 42% of the collagen yield after scanning.

**Table 3.** Effect of mCT on collagen yield, carbon and nitrogen stable isotope ratios/values and atomic C:N ratio. Key: s.d. = standard deviation; c.v. = coefficient of variation; n.a. = not applicable

Sample ID	Subsample	ANU internal lab number N#	Initial pretreatment sample wt (mg)	Pretreatment yield (mg)	Collagen yield (%)	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	C:N ratio
<i>French pig (not scanned)</i>								
FP_LM	1	22890	101	6.13	6.2	4.18	−21.79	3.09
<i>French pig (scanned)</i>								
FP_LM2_A	1	22891	98	4.06	4.0	4.49	−21.95	3.09
<i>Zaglik (not scanned, pretreated at the same time as the scanned samples)</i>								
ZAGLIK	1	21315	293	20.6	7.0	7.77	−19.78	3.23
ZAGLIK	2	21316	269	20.1	7.5	7.76	−19.81	3.22
ZAGLIK	3	21317	248	16.6	6.7	7.81	−19.72	3.22
<i>ZAGLIK average ± 1 s.d. (c.v.)</i>					$7.1 \pm 0.4$ (5.5%)	$7.78 \pm 0.03$ (0.3%)	$-19.77 \pm 0.05$ (0.2%)	$3.22 \pm 0.006$ (0.2%)
<i>Zaglik (not scanned, pretreated at a different time to the scanned samples)</i>								
ZAGLIK	n.a.	18066	494	25.1	5.1	7.70	−19.61	3.21
ZAGLIK	n.a.	18298	621	41.0	6.6	7.52	−19.70	3.21
ZAGLIK	n.a.	18972	490	36.3	7.4	8.03	−19.35	3.21
ZAGLIK	n.a.	19584	512	44.2	8.6	7.73	−19.46	3.21
ZAGLIK	n.a.	19740	538	30.7	5.7	7.58	−19.42	3.21
ZAGLIK	n.a.	20454	557	44.3	8.0	7.58	−19.27	3.20
ZAGLIK	n.a.	21160	576	37.8	6.6	7.85	−19.51	3.20
<i>ZAGLIK average ± 1 s.d. (c.v.)</i>					$6.8 \pm 1.2$	$7.71 \pm 0.18$ (2.3%)	$-19.47 \pm 0.15$ (−0.8%)	$3.21 \pm 0.005$ (0.2%)
<i>Zaglik (scanned)</i>								
ZAGLIK_mCTA	1	21318	271	12.3	4.5	7.23	−19.92	3.25
ZAGLIK_mCTA	2	21319	271	13.4	4.9	7.34	−19.84	3.21
<i>ZAGLIK_mCTA average ± 1 s.d. (c.v.)</i>					$4.7 \pm 0.3$ (6.0%)	$7.29 \pm 0.08$ (1.1%)	$-19.88 \pm 0.06$ (0.3%)	$3.23 \pm 0.03$ (0.9%)
ZAGLIK_mCTB	1	21321	253	10.4	4.1	7.25	−19.88	3.24
ZAGLIK_mCTC	1	21324	273	13.1	4.8	7.36	−19.80	3.22
ZAGLIK_mCTC	2	21325	273	12.1	4.4	7.29	−19.83	3.21
<i>ZAGLIK_mCTC average (± 1 s.d.)</i>					$4.6 \pm 0.3$ (6.1%)	$7.33 \pm 0.05$ (0.7%)	$-19.82 \pm 0.02$ (0.1%)	$3.22 \pm 0.007$ (0.2%)



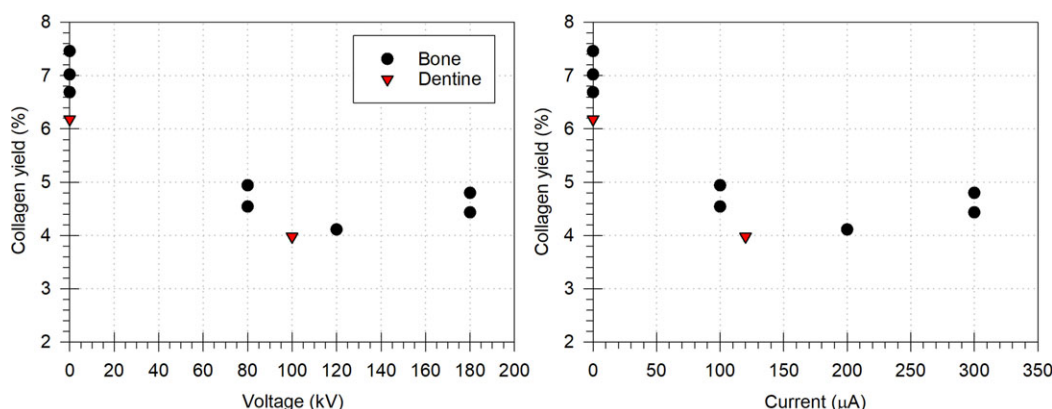
**Figure 1.** Graphical overview of the collagen yield values obtained from the bone and dentine samples. Numerical results may be found in Table 3.

Interestingly, a similar reduction (36%) is independently obtained from the dentine sample, suggesting that mCT scanning may equally impact materials showing somewhat different characteristics (i.e., modern and fossil remains, bones and dental tissues). Additionally, several scanned and unscanned subsamples of the ZAGLICK bone sample were analysed, showing similar variability in collagen yields of about 5.7% to 6.1% (1 standard deviation; Table 3). This illustrates the magnitude of the existing intrinsic uncertainty on the collagen yield results obtained from each fossil specimen. This general uncertainty most likely results from the combined uncertainties associated with the homogeneity of the ZAGLICK bone and the chemical procedure.

Figure 2 shows that there is no strong apparent correlation between the X-ray intensity (expressed in voltage and current) and the collagen yield: while there is a clear drop in the yield after mCT scanning, all mCT scanned subsamples nevertheless return somewhat similar yields, regardless of the voltage (between 80 and 180 kV) and current (100 to 300  $\mu$ A) values employed. Since experimental conditions employed in the present study are similar to those used earlier by Duval and Martín-Francés (2017), the maximum dose absorbed by the samples under high voltage (180 kV) and current (300  $\mu$ Gy) settings may be reasonably estimated to a few tens of Gy. The lower yield obtained for sample ZAGLICK\_B (4.1%) is in agreement with those obtained from the other scanned samples at a 2 $\sigma$  confidence level when considering the existing intra-sample variability (about 6.0% on average at 1 $\sigma$ ; Table 3). Additionally, the values obtained for the dentine samples are comparable with those from the bone (Figure 2).

The drop in collagen yield is unlikely to be caused by heterogeneity of the ZAGLICK bone. Seven crushed aliquots of around 500 mg have been pretreated as part of the routine ANU laboratory throughput. Scatter is likely to reflect the maximum variation expected, as they were taken from various locations scattered across the bone and were processed at different times and by different people. While they vary by about 18.1% (1 s.d.), ranging from 5.1 to 8.6% (Table 3), all values are nevertheless





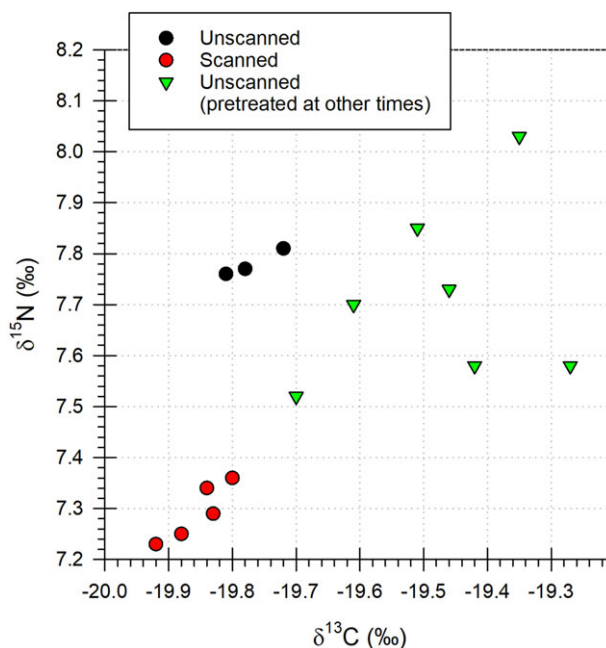
**Figure 2.** Impact of the Voltage (left) and Current (right) values employed during the mCT scanning experiments on the collagen yields. Numerical results may be found in Table 3.

systematically  $> 5\%$ , i.e. higher than the collagen yield measured from the scanned samples (Figure 1).

These observations are compatible with those from Immel et al. (2016) who observed a similar drop in aDNA quantitation after mCT scanning, but no apparent trend in the low dose range ( $< 1,000$  Gy) corresponding to conventional mCT instruments. We hypothesize that the reduction in collagen yield after mCT may be the direct result of radiation damage. Small, damaged fragments of collagen may be either lost in the demineralization stage if soluble, or during ultrafiltration. Additionally, it is possible that minor damage induced by ionising radiation may be amplified during the aggressive acid-base-acid-gelatinization procedure, which is able to hydrolyze peptides, culminating in the removal of a greater portion of the smallest fragments during ultrafiltration. This process is markedly different from aDNA extraction procedures, where soluble components are extracted from bone. Further work is required to confirm this hypothesis.

In these two well-preserved samples, collagen yields remain well above the minimum cut-offs of 0.5 or 1% typically used in radiocarbon laboratories after mCT (e.g., Petchey et al. 2014; van Klinken 1999; Wood et al. 2023). However, the significant reduction of the collagen yield induced by mCT scanning may have two main implications. First, a larger sample may simply be required for radiocarbon analyses from bones and teeth that have undergone previous scanning. Unfortunately, only the most morphologically valuable fossil samples are usually subject to mCT, so this is likely to be problematic. A more prudent approach may be for radiocarbon laboratories to expect lower collagen yields and ensure appropriately-sized standards are processed alongside these samples. If very small samples are extracted containing substantially less than 1mg carbon, precision on the age estimate will decrease. Additionally, samples with originally low collagen yield might also become unsuitable for C-14 dating after mCT scanning, or at least show a yield lower than the usual minimum cut-off value. Consequently, it would be viable to extend the collagen yield quality assurance parameter for mCT scanned bones and teeth and instead focus on C:N ratio as the most appropriate indicator of collagen quality.

However, our results suggest that carbon and nitrogen stable isotopes might also be affected by mCT scanning, although it is important to note that the effect appears very minor (although noticeable), and at most 0.5‰ (Table 3 and Figure 3). There appears to be some heterogeneity within the ZAGLIK bone, with protein extracted at different times to this experiment and from different parts of the bone scattering by 0.5‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios. The three aliquots extracted from the bone at the same time as the scanned samples are more closely grouped and fall within the measurement uncertainty. In contrast, the data from the bone that had been mCT scanned is depleted in the heavy isotope of both carbon and nitrogen, but we see a particularly large shift ( $\sim 0.4\text{--}0.5\text{‰}$ ) in  $\delta^{15}\text{N}$ . No impact is visible on the C:N values, as some scanned subsamples do show somewhat higher ratios, while others return values similar



**Figure 3.** Graphical overview of the effect of mCT scanning on carbon and nitrogen stable isotope ratios obtained on the Zaglik bone sample. Measurement uncertainty of each point is less than  $\pm 0.1\text{‰}$ , at 1 sigma for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios. Numerical results may be found in Table 3.

to the unscanned subsamples (Table 3). The stable isotope composition of bone can vary spatially because the bone is formed at different times during an individual's life. However, the ZAGLIK samples treated alongside the mCT scanned bone were taken from an adjacent location on the bone. Additionally, the mCT scanned samples are clearly distinct from all other subsamples of this bone. Stable isotope results from the pig tooth are less clear as only two samples were analysed. It is possible therefore, that the mCT process has affected the stable isotope values obtained from the bone. This could be expected if different proteins are damaged to different extents during the scanning process. However, given the heterogeneity of the ZAGLIK bone and the very small effect observed, we suggest that this conclusion needs to be investigated further.

### 3. Conclusion

The common assumption in palaeoanthropology that mCT scanning is a non-destructive technique because it usually does not induce any major visible damage to fossils should probably be reconsidered. Our results contribute to a growing body of evidence indicating that there are undoubtedly non-negligible radiation-induced side effects on the fossils. While we do acknowledge the limitations of the present work (small number of samples analysed), our results nevertheless clearly show the systematic impact of routine mCT scanning (i.e., using a conventional instrument and standard acquisition parameters) on the collagen preservation in fossil bones and teeth. In particular, collagen yield and, possibly, stable isotope analytical results, seem to be affected by X-ray exposure. We nevertheless realize that additional mCT experiments will be required in the future in order to properly quantify the variability of this impact and evaluate to what extent its magnitude may depend on the mCT scanning instrument employed, or the geographic origin, geological context, chronology, preservation and/or fossilization degree of the samples analysed.



This is the second study showing that mCT scanning of fossils may have a non-negligible impact on dating results, after a similar work focused on Electron Spin Resonance (ESR) dating (Duval and Martín-Francés 2017). It is the first to suggest that we may see an effect on stable carbon and nitrogen isotope composition, although this is a very minor effect and a more tentative observation that requires further investigations in the future. We therefore recommend caution regarding the systematic and unlimited use of mCT scanning in palaeoanthropology or in any other related disciplines involving fossil material.

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## References

- Bertrand L, Schöeder S, Anglos D, Breese MBH, Janssens K, Moini M and Simon A (2015) Mitigation strategies for radiation damage in the analysis of ancient materials. *Trends in Analytical Chemistry* **66**, 128–145.
- Bailey AJ, Rhodes DN and Cater CW (1964) Irradiation-induced crosslinking of collagen. *Radiat. Res.* **22**, 606–621.
- Bowes JH and Moss JA (1962) The effect of gamma irradiation on collagen. *Radiat. Res.* **16**, 211–223.
- Brock F, Geoghegan V, Thomas B, Jurkschat K and Higham TFG (2013) Analysis of bone “collagen” extraction products for radiocarbon dating. *Radiocarbon* **55**(2), 445–463. doi: [10.1017/S0033822200057581](https://doi.org/10.1017/S0033822200057581).
- Brown TA, Nelson DE, Vogel JS and Southon JR (1988) Improved collagen extraction by modified Longin method. *Radiocarbon* **30**(2), 171–177.
- Duval M and Martín-Francés L (2017) Quantifying the impact of  $\mu$ CT-scanning of human fossil teeth on ESR age results. *American Journal of Physical Anthropology* **163**(1), 205–212. doi: [10.1002/ajpa.23180](https://doi.org/10.1002/ajpa.23180).
- Fallon SJ, Fifield LK and Chappell JM (2010) The next chapter in radiocarbon dating at the Australian National University: Status report on the single stage AMS. *Nucl. Instrum. Methods Phys. Res. B* **268**(7–8), 898–901. doi: [10.1016/j.nimb.2009.10.059](https://doi.org/10.1016/j.nimb.2009.10.059).
- Fox K and Hawks J (2019) Use ancient remains more wisely. *Nature* **572**(7771), 581–583. doi: [10.1038/d41586-019-02516-5](https://doi.org/10.1038/d41586-019-02516-5). PMID: 31462783.
- García-Campos C, Modesto-Mata M, Martín-Torres M, Martínez de Pinillos M, Martín-Francés L, Arsuaga JL and Bermúdez de Castro JM (2020) Sexual dimorphism of the enamel and dentine dimensions of the permanent canines of the Middle Pleistocene hominins from Sima de los Huesos (Burgos, Spain). *Journal of Human Evolution* **144**, 102793. doi: [10.1016/j.jhevol.2020.102793](https://doi.org/10.1016/j.jhevol.2020.102793).
- Griesshaber BM, Osborne DI, Doubleday AF and Kaestle FA (2008) A pilot study into the effects of X-ray and computed tomography exposure on the amplification of DNA from bone. *Journal of Archaeological Science* **35**, 681–687.
- Grün R (2006) Direct dating of human fossils. *American Journal of Physical Anthropology* **131**(S43), 2–48.
- Grün R, Aubert M, Hellstrom J and Duval M (2010) The challenge of direct dating old human fossils. *Quaternary International* **223–224**, 87–93.
- Grün R, Mahat R and Joannes-Boyau R (2012a) Ionization efficiencies of alanine dosimeters and tooth enamel irradiated by gamma and X-ray sources. *Radiation Measurements* **47**(9), 665–668.
- Grün R, Athreya S, Raj R and Patnaik R (2012b) ESR response in tooth enamel to high-resolution CT scanning. *Archaeol Anthropol Sci* **4**, 25–28. doi: [10.1007/s12520-011-0079-7](https://doi.org/10.1007/s12520-011-0079-7).
- Grün R and Stringer C (2023) Direct dating of human fossils and the ever-changing story of human evolution. *Quaternary Science Reviews* **322**.
- Hajdas I, Ascough P, Garnett MH, Fallon SJ, Pearson CL, Quarta G, Spalding KL, Yamaguchi H and Yoneda M (2021) Radiocarbon dating. *Nat Rev Methods Primers* **1**, 62. doi: [10.1038/s43586-021-00058-7](https://doi.org/10.1038/s43586-021-00058-7).
- Hernaiz-García M, Zanolli C, Martín-Francés L, Mazurier A, Benazzi S, Sarig R, Fu J, Kullmer O and Fiorenza L (2024) Masticatory habits of the adult Neanderthal individual BD 1 from La Chaise-de-Vouthon (France). *American Journal of Biological Anthropology* e24926. doi: [10.1002/ajpa.24926](https://doi.org/10.1002/ajpa.24926).
- Immel A, Le Cabec A, Bonazzi M, Herbig A, Temming H, Schuenemann VJ, Bos KI, Langbein F, Harvati K, Bridault A, Pion G, Julien M-A, Krotova O, Conard NJ, Münzel SC, Drucker DG, Viola B, Hublin J-J, Tafforeau P and Krause J (2016) Effect of X-ray irradiation on ancient DNA in sub-fossil bones - Guidelines for safe x-ray imaging. *Scientific Reports* **6**, 32969. doi: [10.1038/srep32969](https://doi.org/10.1038/srep32969).
- Longin R (1971) New method of collagen extraction for radiocarbon dating. *Nature* **230** (5291), 241–242.

- Mandl K, Carlson KSD, Brönnimann D, McCall A, Grassberger M, Teschler-Nicola M, Weiss-Krejci E and Metscher B (2022) Substantiating microCT for diagnosing bioerosion in archaeological bone using a new Virtual Histological Index (VHI). *Archaeological and Anthropological Sciences* **14**(6), 104.
- Marom A, McCullagh JSO, Higham TFG and Hedges REM (2013) Hydroxyproline dating: Experiments on the  $^{14}\text{C}$  analysis of contaminated and low-collagen bones. *Radiocarbon* **55**(2), 698–708. doi: [10.1017/S0033822200057854](https://doi.org/10.1017/S0033822200057854)
- Martín-Francés L, Martínón-Torres M, Martínez de Pinillos M, García-Campos C, Modesto-Mata M, Zanolli C, Rodríguez L and Bermúdez de Castro JM (2018) Tooth crown tissue proportions and enamel thickness in Early Pleistocene Homo antecessor molars (Atapuerca, Spain). *PLoS ONE* **13**(10), e0203334.
- Martín-Francés L, Bermúdez de Castro JM, de Pinillos MM, Martínón-Torres M, Arsuaga JL, Bertrand B and Vialet A (2022) Middle Pleistocene hominin teeth from Biache-Saint-Vaast, France. *Archaeological and Anthropological Sciences* **14**, 215. doi: [10.1007/s12520-022-01680-6](https://doi.org/10.1007/s12520-022-01680-6).
- Martinón-Torres M, d'Errico F, Santos E, Álvaro Gallo A, Amano N, Archer W, Armitage SJ, Arsuaga JL, Bermúdez de Castro JM, Blinkhorn J, Crowther A, Douka K, Dubernet S, Faulkner P, Fernández-Colón P, Kourampas N, González García J, Larreina D, Le Bourdonnec F-X, MacLeod G, Martín-Francés L, Massilani D, Mercader J, Miller JM, Ndiema E, Notario B, Pitarch Martí A, Prendergast ME, Queffelec A, Rigaud S, Roberts P, Shoaee MJ, Shipton C, Simpson I, Boivin N and Petraglia MD (2021) Earliest known human burial in Africa. *Nature* **593**(7857), 95–100.
- Martinón-Torres M, Martín-Francés L, Gracia A, Olejniczak AJ, Prado-Simón L, Gómez-Robles A, Lapresa M, Carbonell E, Arsuaga JL and Bermúdez de Castro JM (2011) Early Pleistocene human mandible from Sima del Elefante (TE) cave site in Sierra de Atapuerca (Spain): A palaeopathological study. *Journal of Human Evolution* **61**, 1–11.
- Olejniczak AJ and Grine FE (2006) Assessment of the accuracy of dental enamel thickness measurements using microfocal X-ray computed tomography. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* **288A**(3), 263–275.
- Petchey F, Spriggs M, Bedford S, Valentin F and Buckley H (2014) Radiocarbon dating of burials from the Teouma Lapita cemetery, Efate, Vanuatu. *Journal of Archaeological Science* **50**, 227–242. doi: [10.1016/j.jas.2014.07.002](https://doi.org/10.1016/j.jas.2014.07.002).
- Rahman N, Khan R and Badshah S (2018) Effect of x-rays and gamma radiations on the bone mechanical properties: literature review. *Cell Tissue Bank* **19**, 457–472. <https://doi.org/10.1007/s10561-018-9736-8>.
- Richards GD, Jabbour RS, Horton CF, Ibarra CL and MacDowell AA (2012) Color changes in modern and fossil teeth induced by synchrotron microtomography. *Am. J. Phys. Anthropol.* **149**, 172–180. <https://doi.org/10.1002/ajpa.22103>.
- Sauer K, Zizak I, Forien JB, Rack A, Scoppola E and Zaslansky P (2022) Primary radiation damage in bone evolves via collagen destruction by photoelectrons and secondary emission self-absorption. *Nat. Commun.* **13**, 7829. <https://doi.org/10.1038/s41467-022-34247-z>.
- Smith TM, Tafforeau P, Reid DJ, Pouech J, Lazzari V, Zermeno JP, Guatelli-Steinberg D, Olejniczak AJ, Hoffman A, Radović J, Makaremi M, Toussaint M, Stringer C and Hublin J-J (2010) Dental evidence for ontogenetic differences between modern humans and Neanderthals. *Proceedings of the National Academy of Sciences* **107**(49), 20923.
- Stuiver M and Polach HA (1977) Discussion reporting of  $^{14}\text{C}$  data. *Radiocarbon* **19**(3), 355–363. doi: [10.1017/S0033822200003672](https://doi.org/10.1017/S0033822200003672).
- Tafforeau P, Boistel R, Boller E, Bravin A, Brunet M, Chaimanee Y, Cloetens P, Feist M, Hoszowska J, Jaeger JJ, Kay RF, Lazzari V, Marivaux L, Nel A, Nemoz C, Thibault X, Vignaud P and Zabler S (2006) Applications of X-ray synchrotron microtomography for non-destructive 3D studies of paleontological specimens. *Applied Physics A* **83**(2), 195–202.
- Tafforeau P and Smith TM (2008) Nondestructive imaging of hominoid dental microstructure using phase contrast X-ray synchrotron microtomography. *Journal of Human Evolution* **54**(2), 272–278.
- van Klinken GJ (1999) Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science* **26**(6), 687–695. doi: [10.1006/jasc.1998.0385](https://doi.org/10.1006/jasc.1998.0385).
- Wood RE, Esmay R, Usher E and Fallon SJ (2023) Sample preparation methods used at the Australian National University radiocarbon facility. *Radiocarbon* **65**(2), 573–589. doi: [10.1017/RDC.2022.97](https://doi.org/10.1017/RDC.2022.97).
- Yu W, Herries AIR and Joannes-Boyau R (2022) Using X-rays as an irradiation source for direct ESR dating of fossil teeth. *Quaternary Geochronology* **72**, 101372.
- Zanolli C, Biglari F, Mashkour M, Abdi K, Monchot H, Debue K, Mazurier A, Bayle P, Le Luyer M, Rougier H, Trinkaus E and Macchiarelli R (2019) A Neanderthal from the Central Western Zagros, Iran. Structural reassessment of the Wezmeh 1 maxillary premolar. *Journal of Human Evolution* **135**, 102643. doi: [10.1016/j.jhevol.2019.102643](https://doi.org/10.1016/j.jhevol.2019.102643).