*Please cite this as: Haponava, V., Fibiger. L., Butler, I.B. and Pickard, C. 2025 Towards Responsible Destructive Analysis: A guide to the recording of archaeological tooth samples with laboratory process visualisation, Internet Archaeology 69. https://doi.org/10.11141/ia.69.4*

# Towards Responsible Destructive Analysis: A guide to the recording of archaeological tooth samples with laboratory process visualisation

*Vera Haponava, Linda Fibiger, Ian B. Butler and Catriona Pickard*

With increasing use of destructive analysis in archaeology, a tension has arisen between the need to preserve osteological collections for future generations and to use them now for the public benefit of knowledge generation. Existing guidelines mostly address curatorial issues, or focus on pre-sampling steps, rather than presenting protocols that can assist researchers in being more responsible regarding invasive analysis and the preservation of osteological collections. This article therefore presents guidelines for the recording of archaeological tooth samples prior to destructive analysis in the form of a process diagram accompanied by written instructions. The aim is twofold: to promote good practice in preserving a record of a sample before its destructive analysis, and to provide accessible material that can be used in lectures or training to assist students in visualising common laboratory processes in the field of bioarchaeology, as well as for public outreach and knowledge exchange. The diagram is enriched with laboratory videos for each step, and should help demystify the laboratory process for general audiences.

## 1. Introduction

Anthropological collections are an important scientific resource for the study of past populations. Recently, concerns have been raised about the exponential increase in the pace of destructive sampling, highlighting archaeological remains as a finite and irreplaceable resource (Austin *et al.* 2019; Hendy *et al.* 2018; Mays *et al.* 2023; Pálsdóttir *et al.* 2019; Roberts 2016; Squires *et al.* 2019). This creates a tension between “the duty to protect and preserve archaeological heritage” (Pálsdóttir *et al.* 2019 , 3), including the integrity of skeletal remains (Squires *et al.* 2019), and the potential of new knowledge generated without waiting for more advanced methods to emerge. Some biomolecules continue to deteriorate over time, and osteological collections themselves are subject to damage and loss, both physical and of the contextual information related to them (Antoine and Ambers 2014; Mays *et al.* 2023;

Pálsdóttir *et al.* 2019). Consequently, there have been calls for the development of best practice (Austin *et al.* 2019) and for wider discussion and debate concerning destructive analysis (Squires *et al.* 2019).

Common destructive methods used in archaeology include ancient (a)DNA analysis, stable isotope analysis, radiocarbon dating, proteomics and microscopy (Mays *et al.* 2023; Squires *et al.* 2019). Various guidelines have been already developed regarding the practical aspects of sampling and analyses, covering such topics as handling human remains (Brickley and McKinley 2004), sampling human remains for aDNA (Elders *et al.* 2011), methodology and reporting for stable isotope analysis (Roberts *et al.* 2018), and the generation of ancient protein data (Hendy *et al.* 2018) in archaeology. Detailed protocols describe various aspects of these analyses, such as the laboratory procedures for collagen extraction (Richards and Hedges 1999; Sealy *et al.* 2014), incremental sampling of dentine (Beaumont *et al.* 2013; Curtis *et al.* 2022; Czermak *et al.* 2020), sampling calculus (Sabin and Yates 2020), pre-treatment of enamel bioapatite (Balasse 2002; Ventresca Miller *et al.* 2018), and sampling for aDNA (Parker *et al.* 2021), including instructional videos (Parker *et al.* 2021; Ventresca Miller *et al.* 2018). It is clear that similar guidelines and protocols are necessary to assist researchers in the responsible sampling and preservation of osteological collections.

Multiple studies and institutions have pointed out the need to properly document samples before destructive analysis (Antoine and Ambers 2014; BABAO 2019; Eriksson 2013; Human Remains Working Group 2013; Mays *et al.* 2023; Smithsonian National Museum of Natural History 2018; Squires *et al.* 2019). However, their recommendations tend to be directed at curating institutions or focus on the steps to be taken before the sampling or analysis itself (Austin *et al.* 2019; Human Remains Working Group 2013; Mays *et al.* 2023; Pálsdóttir *et al.* 2019). Curating institutions are tasked with regulating access to samples, to preserve collections for future generations of researchers. For example, the British Association for Biological Anthropology and Osteoarchaeology (BABAO), the Advisory Panel on the Archaeology of Burials in England (APABE) and the German Museums Association advise that the use of destructive methods only be allowed when non-destructive methods cannot yield the answers to research questions. Their guidelines suggest taking only what is necessary; minimising sample sizes and extracting them in an unobtrusive manner; performing any analyses that might require integrity of the skeletal parts (e.g. age, sex and pathological assessments) before sampling; undertaking sampling that allows the same sample, such as a tooth, to be used for multiple analyses; targeting teeth that have their antimeres (the same tooth type from the opposite side of the jaw) available; and returning remnants of samples to the host institution (BABAO 2019; Brickley and McKinley 2004; Elders *et al.* 2011; Human Remains Working Group 2013; Mays *et al.* 2023). Similar recommendations and other suggestions, such as running a feasibility study on a smaller dataset before extending destructive sampling to a larger dataset, are provided elsewhere (Hendy *et al.* 2018; Roberts 2016; Squires *et al.* 2019). However, from the perspective of someone undertaking or planning a study involving invasive sampling, what does it mean to ‘properly document’ the sample? Specific steps to mitigate the impact of destructive sampling, as proposed in the literature, include:

- creating a written record (database) of morphological features and measurements before extracting the sample (Antoine and Ambers 2014; BABAO 2019), such as noting down the presence of secondary and tertiary dentine, cementum layers, tooth decay and attrition in teeth (Czermak *et al.* 2020), as well as standard measurements and weighing archaeofaunal samples (Pálsdóttir *et al.* 2019)

- photographing the sample from different angles, as well as the sampled area before and after sampling (BABAO 2019; Czermak *et al.* 2020; Mays *et al.* 2023; Pálsdóttir *et al.* 2019; Squires *et al.* 2019)
- making impressions and casts, particularly when sampling tooth crowns (Antoine and Ambers 2014; BABAO 2019; Czermak *et al.* 2020; Mays *et al.* 2023; Squires *et al.* 2019)
- if possible, creating a virtual copy in three-dimensional (3D) format using photogrammetry, 3D scanning, (micro-) computerised tomography (CT)-scanning, X-ray or magnetic resonance tomography (MRT), which would allow for subsequent 3D modelling, again more commonly advised for teeth (Antoine and Ambers 2014; BABAO 2019; Czermak *et al.* 2020; Human Remains Working Group 2013; Mays *et al.* 2023; Pálsdóttir *et al.* 2019; Squires *et al.* 2019)
- keeping spare tissues or adherent substances for future analyses, for example dental calculus in the case of teeth (Czermak *et al.* 2020) and enamel from sections (Avery *et al.* 2023), and inventorying the leftover material (Pálsdóttir *et al.* 2019).

In summary, existing guidelines focus on pre-sampling steps (such as the project design, justification and obtainment of permissions) and actions to be taken by curating institutions, while those that discuss how to minimise the negative impact of destructive analysis before and during sample processing do so in brief, high-level, steps. The first aim of this article, therefore, is to suggest how a digital and physical record of a tooth sample can be created before destructive analysis, based on an example from a stable isotope project that incorporated the steps already proposed in the literature. Teeth are considered to be particularly informative parts of the human or animal body compared to a bone sample of the same size, while stable isotope analysis is one of the most common destructive analyses undertaken in archaeology (Mays *et al.* 2023; Squires *et al.* 2019). Every tooth contains a unique chronological record of development, because its tissues are not remodelled later in life (Hillson 1996), and a high concentration of metric, non-metric and pathological features relative to its size. While it is necessary to document a bone sample by creating a record of all its observable features that may be related to sex or age identification, non-metric traits and pathologies, other procedures (such as taking casts and X-rays) may be deemed less necessary unless the bone has unique features. Tooth samples, therefore, are well suited for exemplifying an extended sample recording process.

Unlike the codes and guidelines reviewed above, this article focuses on the steps that can be taken once permissions for destructive analysis and access to the samples have been obtained. Several methods for creating digital and physical records of a tooth sample are described in how-to sections, supplemented with advice from the literature, and incorporated into the flow of a common laboratory process in the form of a diagram. This example can be used by researchers planning to undertake similar destructive analyses and following the corresponding recommendations found in, for example, BABAO or APABE codes of practice, and to design research proposals that have a higher likelihood of being approved by institutions curating osteological collections, such as museums.

The second aim of this article is to provide a visualisation of the proposed steps within a larger picture of common laboratory processes. This is done in the form of a process diagram implemented in Prezi and enriched with short videos. The videos are not meant to be detailed how-to videos for training in each specific step, such as those, for example, in Ventresca Miller *et al*. (2018) and Parker *et al*. (2021); rather they represent high-level illustrations that can be used to present common laboratory processes to someone not familiar with them, and possibly not intending to personally reproduce them. The intended audience includes practitioners such as archaeologists (who send

their material to laboratories and would benefit from a general overview of what happens to their samples), students, and the lay public with a general interest in scientific analysis.

## 2. Pre-analysis Recording of How-To

A full diagram of the sample preparation and laboratory analysis is shown in Figure 1. The example used includes the preparatory steps, which are described in detail in this and the following section, as well as the dentine collagen and enamel carbonate processing steps, which follow the protocols used at the Isotope Laboratory, Max Planck Institute of Geoanthropology (Jena, Germany). The majority of the preparatory steps were carried out at the Andy Barlow Laboratory, University of Edinburgh (Edinburgh, UK). The samples used were archaeological human teeth from Belarus. Permissions were obtained from the source institutions for the use of the samples, and ethical approval for the project was received from the History, Classic and Archaeology School Ethics Committee at the University of Edinburgh (University of Edinburgh 2024).

[Interactive figure. ONLINE ONLY]
Figure 1: Diagram of the sample preparation process, including the steps for preserving a record of the sample and unused tissue (highlighted in bold), and collagen and carbonate processing. [Download image].

The process illustrated in Figure 1 covers the taking of incremental samples from human tooth dentine and bulk enamel samples. The method used is slightly different to the analysis of bulk collagen from dentine, and is based on Czermak *et al*. (2020). The more recently developed incremental dentine sampling methods are generally considered to be less destructive, as they allow the preservation of at least part of the physical sample for further analyses (Cheung *et al.* 2022; Czermak *et al.* 2020), although views on this differ (Squires *et al.* 2019). However, this analysis remains, inevitably, destructive: it leads to a loss of the physical integrity of the tooth, including information about the shape of the crown and roots, of any lesions/cavities, non-metric traits, and the topography of the occlusal surface (cusps, furrows) and other surfaces (e.g. enamel hypoplasias or other lesions, such as hypocalcification). Various features may become unobservable, such as calculus deposits, enamel and dentine mass, taphonomic features, and information about the position of the tooth relative to other teeth or to the alveolar process (e.g. the distance between the edge of alveolar bone and dentine–enamel junctions). Some of these are lost during extraction of the tooth from the mandible or maxilla, and others later on during cutting, drilling, crushing and chemical processes such as demineralisation. Because incremental sampling is a more complicated process than bulk sampling of dentine, it provides the opportunity for a more detailed illustration of how various tissues of a tooth can be preserved in digital or physical form at different stages of the process.
Two sets of steps related to sample recording and the preservation of spare tissues are highlighted in bold in Figure 1, and are described in detail in sections 2 and 3. This is not an exhaustive list of all possible measures, but a recommended minimum. The rest of the steps in Figure 1 relate to routine sample preparation and analyses, in this case collagen and carbonate processing.

### 2.1 Collection and cleaning of the sample (steps 1–3)

Where possible, the sampled area (e.g. the mandible or maxilla) should be photographed before (and after) extracting a tooth. The teeth in our example were cleaned using an ultrasonic bath for 20–30 min with one change of MilliQ (ultra-purified, effectively carbon-free) water, adding extra washes if needed. Cleaning methods vary

among laboratories; some common alternatives used in biomolecular analysis include sandblasting or gentle abrasion of the outer surface with a drill (Beaumont *et al.* 2013; Ventresca Miller *et al.* 2018), which may result in obliteration of micro-features of the enamel surface. In dental anthropological research, gentler methods, like the use of soft brushes or cotton swabs soaked with solvent, are preferred (Fiorenza *et al.* 2009; Hillson 1992).

**2.2 Catalogue (step 4)**

While a written record of some features (e.g. pathological lesions) is inevitably interpretative and thus may be less useful than a replica or an image, it is an accessible way to provide general information. A digital catalogue of samples (e.g. in the form of a table or relational database) allows all related information to be connected, from archaeological context to morphological features and the history of treatments (e.g. cleaning, fragmentation, X-ray exposure, contact with chemicals) applied to them. The need for physical and digital long-term storage for all types of research output and surplus material should be considered at the beginning of the project, and a sample catalogue is indispensable for keeping record of the outputs and their locations. Links to digital data related to the sample can be added to its associated record; for sample parts or replicas, an indication of their physical location should be kept.

As a minimal written record for each tooth, the following were noted for our Belarussian samples: attrition according to Smith (1984), Moorrees development stages (1963a,b, cited in AlQahtani *et al.* 2010) and pathologies such as caries stages defined by Hillson (2001), enamel hypoplasia (Brickley and McKinley 2004; Steckel *et al.* 2018), and dental calculus stages defined by Hillson (1996). A written record of the samples was catalogued in a spreadsheet using Microsoft 365 Excel. The history of treatment was included for each tooth (see examples in sections 2.3 and 2.4). Sub-samples obtained during the process (such as of enamel, calculus and dentine) were added to the catalogue with their own identification number and relevant additional characteristics, such as sample weight or serial number and the anatomical location of the dentine increments (e.g. the crown or root). The latter may be useful for improving the accuracy of the assignment of chronological age to dentine increments, as the dentine formation rate varies between crown and root (Beaumont *et al.* 2013).

In the example reported here, limitations to recording the samples were imposed by prior collection of samples for a separate project (Haponava *et al.* 2022). However, where possible, it is advisable to record tooth characteristics prior to extracting it. In particular, the distance between the alveolar bone and the cemento-enamel junction (CEJ) (which may be used as a measure of alveolar resorption) should be recorded before extracting a tooth. Other characteristics listed here should hopefully survive and remain observable in teeth isolated from the jaw. In cases of poor preservation, however, a tooth may be damaged during extraction such that some of its features are rendered unobservable, for example calculus may often fall off, or enamel crumble, during extraction.

A wider variety of tooth characteristics could be recorded, such as the tooth's dimensions and non-metric traits, later developing tissues such as secondary and tertiary dentine, and cementum layers. More methods for recording tooth morphology and pathology can be found elsewhere (Hillson 2008 , 2023). When the specific requirements of further research are uncertain, it is obviously impossible to compile an exhaustive catalogue of tooth features. Hopefully, however, in most cases the methods described further below (casts, photographs and micro-CT scans) should be sufficient to reconstruct adequately lost features of the tooth. Any written record should be adapted to the time and resource constraints of the given project, and should focus on features for which there is reason to believe that photographs or CT scans may not reflect the information adequately.

### 2.3 Casts (step 5)

As mentioned, a written record arguably cannot foresee all possible future needs. It also does not allow for the same degree of interaction with the material as physical evidence (Brickley and McKinley 2004), making it necessary to preserve some forms of the latter. Common types of replication for teeth are impressions or moulds (negative images of a surface), from which casts (positive images) can be made. For impressions, silicone materials are recommended because of their dimensional stability, and epoxy resin (which can be coloured with a compatible die) is commonly used for casting a replica (Fiorenza *et al.* 2009; Galbany *et al.* 2004; Hillson 1992). Latex also performs well for high-resolution moulds/impressions, and provides a cost-effective alternative to silicone. Impressions and replicas should be stored in clean and sealed separate containers (such as sample tubes or bags), to prevent contact with dust and any mechanical damage (Hillson 1992). Recommendations on choosing a replication strategy (with evaluation of the achieved resolution of the replica, its dimensional stability and speed of production) and guides on handling procedures can be found in Hillson (1992), Galbany *et al.* (2004), Fiorenza *et al.* (2009).

In our example, latex moulds of tooth crowns were created using MBFibreglass Polycraft Liquid Latex, a material that was already available in the laboratory. The latex provided a high-precision copy of the tooth surface topography; however, as this natural material contains proteins, its use is not advisable if ancient protein analysis is planned (Hendy *et al.* 2018). Where possible, we sampled any calculus before making the moulds, to preserve it for potential future analyses, including proteomics.

To make the mould, each tooth was inserted in plastic foam to approximately the CEJ level, to keep it stable. The latex for the initial layers was diluted slightly with deionised water (MilliQ), to achieve higher liquidity and thus a more precise imprint of the tooth. Five to six such layers were applied to the crown with a brush, allowing each layer to dry before applying the next one. The brush was frequently washed in water with added washing-up liquid, to avoid the latex on it drying and sticking the bristles together. Care was taken to eliminate bubbles, especially in the first layer. The initial 5–6 layers were then left to dry for a day. Subsequently, a further 5–6 layers of undiluted latex were applied and left to dry for another day. Finally, latex was mixed with a thickener, in general following the manufacturer's guidelines, the thickener representing 5% of the mixture or added until the mixture reached the desired stiff consistency, i.e. it retained its form. This thick latex mixture was applied generously to the crown over the initial latex layers to make an external case for the mould. It was then left for a day or more, until fully dry. Once dry, the moulds were removed from the teeth. A 2:1 mixture of Buehler EpoThinTM 2 Epoxy Resin and EpoThinTM 2 Epoxy Hardener was prepared, in general following the manufacturer's guidelines, in a paper cup, adding a small amount of white dye to produce casts of a colour similar to the tooth crown surface. When the mixture warmed up (indicating the chemical reaction had started), the resin was poured into the moulds quickly but carefully, to avoid air voids. Exerting light pressure on the moulds with freshly poured resin can help get rid of any bubbles. The casts were left to set in the moulds for 1–2 days or until fully solid, then taken out. If a completed cast appeared to have faults, the cast and/or the mould was remade, depending on the cause of the imperfection. Ideally, making multiple replicas from the same impression should be avoided, as it results in the loss of surface detail (Galbany *et al.* 2004). Both the completed cast and the mould were put in labelled plastic bags and kept as a physical record of the sampled tooth.

Teeth with deep cavities (e.g. caries) presented a challenge when making the replicas, as it was hard to penetrate the cavity completely, to its floor level, with latex. Additionally, it may not be advisable to aim for such penetration, as it could potentially cause

contamination with organic material. Therefore, certain analyses that could have been carried out on the original teeth, for example measuring the depth of a caries cavity or observing the morphology of its floor, are unlikely to be accurate if attempted on a cast, and require other types of replication, such as a high-resolution 3D scan.

**2.4 Tooth photographs and micro-CT scans (steps 6–7, 12 and 16)**

Photographic images allow the preservation of colour information, which is not possible with other imaging techniques, such as CT, the output of which is a 3D array of grey values. Photographs should be made at every processing stage and from multiple aspects, with additional photographs focusing on specific features such as pathological changes where visible. In all cases a photographic scale with a colour chart and a label with the sample ID should be included in the photo. For better illustration of smaller features of the surface (e.g. non-metric traits, developmental stage and attrition), taking macro-photographs with a digital single lens reflex or mirrorless camera mounted with a macro-lens is recommended (Hillson 2023). Where resources permit or the uniqueness of the material requires extra recording, producing images with a scanning electron microscope should be considered, as it allows the capture of fine crown surface features such as perikymata, microwear and hypoplastic lesions (Galbany *et al.* 2004; Hillson 1992; 2023).

The video-recorded process shows photographs taken from six aspects (buccal, mesial, distal, lingual, occlusal and root sides), with a Canon EOS 800D or a smartphone Samsung Galaxy A53 5G camera. Additionally, photographs of the cut section (from two sides) and of the demineralised section after punching were taken.

Micro-CT scans provide a record of the tooth's inner structure and 3D shape. Information on scanner and image reconstruction settings, sufficient to enable the tomographic data to be reconstructed using different settings at a later date if required, should be added to the sample catalogue. Particularly when sampling fossil remains, the potential impact of intense X-ray exposure (e.g. in synchrotron micro-CT) on the preservation of aDNA molecules should be considered, and the scanning strategy adapted accordingly (see Immel *et al.* 2016).

Micro-CT scans in our example were made using a bespoke scanner designed and built in the Experimental Geoscience Facility of the School of Geosciences, University of Edinburgh. It comprises a Feinfocus 10-160 keV dual transmission and reflection X-ray source, a MICOS ultra high precision air-bearing rotary sample table, and a Perkin Elmer XRD0822 20x20 cm, 1 megapixel amorphous silicon flat panel X-ray camera with a terbium-doped gadolinium oxysulfide scintillator. For stability during scanning, each tooth was set into floral foam. Data acquisition software was written in-house, and data reconstruction was done by filtered back projection using Octopus Reconstruction v8.9 software, using the same settings for all samples. The scanner and reconstruction settings were added to each tooth's record, and included the X-ray energy, target power loading, distance between source and detector and between source and object, number of projections, exposure time, energy filter, number of 'offset' and 'gain' (dark and flat) images, and the voxel size of scanned images. Voxel (a 3D counterpart to a pixel) can be used as a scale to determine the actual (metrical) size of a scanned object. Voxel sizes in this case study ranged from 22 to 26 µm, compatible with the resolution required in recent research in dental anthropology (Coutsiers Morell *et al.* 2022; Hillson 2023).

The scanning time for each tooth was ~0.5h, and the storage size of 2000 projections acquired per each scan was 4GB. For long-term-storage, Edinburgh DataVault 10-year retention was used, which backs-up data to three widely separated locations and provides a DOI to make it findable (e.g. Haponava 2025). Where in-house solutions are not available, storage may be outsourced to external repositories such as the UK's Archaeology Data Service.

### 3. Preservation of Spare Tissues for Future Study

Often, a sample taken for destructive analysis contains more tissue than is required. It should be discussed in advance with the curating institution what sample components should be returned, as in many cases it is best to keep the derived products (e.g. tooth or bone powder, collagen and DNA extracts) in the laboratory that performed the analysis, which should have the necessary facilities to store such material (Pálsdóttir *et al.* 2019). As leftover components may be used in further analyses, it is crucial to catalogue them, indicating the location of their storage, to ensure accessibility for future research.

In our incremental sampling process, not all parts of the tooth were required for stable isotope analysis. In particular, calculus and parts of the enamel that adhere to the cut section would be lost during demineralisation if not separated beforehand. Although cutting sections for incremental sampling usually leaves unused parts of the tooth suitable for further study, further research may in fact still be hampered. As previously mentioned, making latex moulds could render calculus unsuitable for the analysis of proteins, even where it does not fall off in the process of cutting the tooth. Likewise, cutting the tooth to obtain the tooth section and incremental samples might make the remaining part of the tooth unusable for other study methods such as aDNA analysis, because of, for example, contamination. To minimise such loss, extra steps were taken to ensure that the parts of the tooth that were not required for the stable isotope study remained usable for other analyses (refer to the 'Preserve spare tissues' in Figure 1). Calculus was sampled following the protocol of Sabin and Yates (2020) with slight adjustments, aiming to preserve it not only for stable isotope analysis, but also for proteomic analysis. If the sampled calculus had previously come into contact with latex during mould-making, a corresponding note was made on the sample bag and in the catalogue. Where two teeth of the same individual contained a visibly substantial quantity of calculus, they were sampled separately. Where the quantity of calculus was visibly small on at least one of the two teeth (e.g. a tiny patch), samples from both teeth of the same individual were combined.

Using a precision saw that rotates through a water bath for cutting tooth sections introduces a level of contamination into the pulp cavity, which would make the remaining parts of the tooth unsuitable for aDNA analysis. For that reason, where several non-fused roots were available, a root was cut from each tooth following a protocol for preparing a tooth sample for aDNA based on Keller and Scheib (2023) and Parker *et al*. (2021). The specific steps and equipment used will depend on the laboratory: for example, for our study no dedicated aDNA facility or clean room was available, so the general bone chemistry laboratory and equipment were used. DNA Exitus and ultra-violet (UV) irradiation were not available, so for cleaning 12% bleach was used to wipe down all the working surfaces and soak the drill discs and brushes.

The unused parts of a cut tooth usually preserve most tissues, including enamel, for future study. However, some teeth (e.g. lower incisors, and worn or poorly preserved teeth) may have little enamel left, making retention of as much enamel as possible highly desirable, to enable the analysis of, for example, carbonate or phosphate for oxygen, carbon and strontium isotopes. Therefore, before the cut sections were put in acid for demineralisation, enamel was drilled off from those sections that had a substantial quantity of it, and that appeared robust enough to not be damaged by drilling. In the case of third molars, such enamel was used for the stable isotope analysis of carbonate, with the addition of enamel from unused tooth parts where a section did not yield 10 mg of powder, and preserving any enamel in excess of the 10 mg needed for the analysis of carbon and oxygen isotopes. An example of a protocol for enamel drilling can be found in Ventresca Miller *et al*. (2018).

## 4. Limitations

The diagram presented in Figure 1 remains a simplified depiction of a process that in practice flows in a less orderly fashion. The sequence of steps described in written form does not perfectly align with the diagram. For example, it was noted for this incremental sampling example that calculus was sampled where possible before creating casts, to avoid contamination. In fact, calculus fell off at various stages of the process, starting with the extraction of the tooth from its socket, through to its handling and cleaning. The same was true for enamel in some of the less well-preserved teeth, so both calculus and enamel were in reality collected at multiple stages. Additions to the written catalogue were also made at multiple stages, although only one step for cataloguing is included in the diagram in order not to overcomplicate it. Therefore, the diagram should not be taken literally, but rather as a high-level guide to a flexible and complex process.

Time and cost constraints may not permit the same level or manner of recording in all cases. Some projects may require a more elaborate recording or preservation plan, stemming from the perceived uniqueness of the samples or guided by the planned methodology. For example, cementum may be separated from tooth samples before demineralisation. Which steps to take or to omit remains a matter of discretion within an individual project. These steps are time-consuming and should be included in the project schedule and budget planning to account for the necessary consumables and equipment. The cost of long-term storage should also be considered, including physical and digital storage for different types of output. Micro-CT scans in particular may require hundreds of gigabytes of long-term digital storage space, doubled by back-ups of the data, which should not be stored in the same location as the original (e.g. hard drive backups should be in different buildings, or a reliable online repository used). However, creating a high-resolution, precise, 3D copy of a sample before destruction may overcome many of the faults of insufficient or biased written and visual records, and future proof for later research needs.

Although the steps reviewed and proposed in this article are aimed at enhancing the preservation of various types of information for a destructively analysed sample, each may have its own implications that need to be carefully considered. For example, casts or imprints of teeth are more accessible than CT-scans, to many researchers and probably anyone who is willing to examine the tooth but lacks specialist knowledge in CT or 3D modelling. However, using certain materials like latex for the creation of moulds reduces the usefulness of the remaining tooth parts or calculus for certain kinds of biomolecular analysis, such as of ancient proteins.

## 5. Process Visualisation

The steps aimed at sample preservation, as described above, as well as the further steps of common collagen and carbonate stable isotope analyses, are also presented in the form of short videos incorporated into an interactive diagram implemented in Prezi. The videos were filmed on a smartphone Samsung Galaxy A53 5G camera and edited using VideoPad v 16.46 by NCH Software.

The latter part of the process is only briefly described here as it followed laboratory-specific protocols. In short, the tooth was immersed in Herculite for stability, then cut with a precision saw to obtain a mid-section. This longitudinal tooth section was demineralised in 0.5M HCl until a flexible ‘pseudomorph’ of the original hard section remained. Further, sequential, micro-samples were obtained from crown cusp to root apex using a disposable biopsy punch with plunger, as described in Czermak *et al*. (2020). The punched dentine samples were freeze dried, weighed into tin capsules, and combusted in an elemental analyser (EA) coupled to a mass spectrometer, to obtain ratios between heavy and light isotopes of carbon and nitrogen usable for quantitative analysis.

For the acquisition of carbonate samples, enamel was drilled with a diamond-tipped Dremel drill and pretreated following a protocol similar to the one described in Ventresca Miller *et al*. (2018). The enamel powder was washed in acetic acid, rinsed with MilliQ water, and freeze dried. The dried sample was weighed into borosilicate glass vials that were flush filled with helium, after which phosphoric acid was added to the samples. The gases produced in the reaction of the acid with the samples were analysed for stable carbon and oxygen isotopic composition using a gas bench connected to a mass spectrometer.

[Presentation - ONLINE ONLY]

*The laboratory process for stable isotope analysis of dentine, including steps for sample preservation, and collagen and carbonate stable isotope analyses.*

The diagram with accompanying short videos in the presentation is intended to serve as an illustration of common laboratory processes for a wider audience, and may be particularly useful in a classroom setting as a visual resource. The Prezi version (above) can be accessed online through a browser, and does not require an account or installation of any additional software. The Prezi version can be navigated forwards and backwards like a sequence of slides in a presentation, but also more freely by clicking on any step in the process that the user wishes to navigate to. Additionally, the same presentation is provided in the form of a recorded video (below), which may be played online or downloaded.

[Video - ONLINE ONLY]
Video: A video showing the steps described in the presentation: sample preservation, and collagen and carbonate stable isotope analyses. The video was recorded using Debut (NCH Software) and Prezi for Desktop applications (No audio).

## 6. Conclusion

This article provides guidelines for recording archaeological tooth samples prior to destructive analysis, in order to mitigate the impact of material loss. The process diagram, accompanied with written instructions, should provide practitioners with an example of good practice that can help make their invasive analysis design more responsible regarding the preservation of osteological collections, and therefore more acceptable to curating institutions. An interactive version of the same diagram, enriched with short videos, was produced to provide accessible material that can be used in lectures or training, to visualise common laboratory processes in the field of bioarchaeology, as well as for public outreach and knowledge exchange, to demystify the laboratory process for general audiences.
In the context of accelerating use of destructive analyses in archaeology, we hope that the discussion, publication and dissemination of standards for physical and digital documentation of samples will similarly increase in quantity to address our duty to steward and preserve archaeological remains for future generations of researchers. The steps presented here may represent additional effort; however, if they are implemented successfully, the added value of such research is much more likely to outweigh the negative impact of the destructive analysis.

## Acknowledgements

The authors express their gratitude to two anonymous reviewers for their valuable comments and suggestions; to Dr Emma Smith and Kris Walker from the University of Edinburgh, and Erin Scott from the Max Planck Institute of Geoanthropology, for the

training and support provided in the lab; to Dr Elżbieta Jaskulska (University of Warsaw) and Erin Scott for the provision of photos; and to the School of History, Classics and Archaeology, and the School of Geosciences, of the University of Edinburgh and the Max Planck Institute of Geoanthropology, for the provision of materials, instrumentation and lab space. We would like to thank our colleagues from Połack State University, Belarus, and the Institute of History, National Academy of Sciences of Belarus, for the initial provision of samples that were used in this project.

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