Least Destructive Rapid Scanning of Human Teeth to Test Their Suitability for U-Series Analysis

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ABSTRACT

Excavations in the Grotte de la Chèvre have taken place since the 19th century and yielded more than 100 human specimens together with numerous artifacts from the Middle Paleolithic, Upper Paleolithic, and more recent times. Unfortunately, due to considerably different excavation standards in earlier excavations, none of the specimens can be securely provenienced. Some of the remains may represent oldest humans fossils found in historic Brittany. This can only be confirmed through direct dating. On faunal materials, U-series dating is usually carried out a series of analyses along a cross-section profile, which necessitates the cutting of the specimen. Here, we present a rapid scanning method, which allows the assessment of whether a sample is actually suited for U-series dating with minimal sample damage. Laser ablation ICP-MS was used for the analysis of U-series isotopes close to the surface of the roots of two teeth from the Grotte de la Chèvre. The laser analysis created pits with a diameter of about 200µm and a depth of about 100µm. This allowed the assessment of the ^{230}Th concentrations close to the surface of the dentine where the oldest apparent U-series ages are expected. In the first sample, all isotopic concentrations were too low for the calculation of meaningful U-series results, while the second sample yielded very low ^{230}Th/^{238}U activity ratios, indicating a recent age of perhaps a few thousand years. Consequently, both teeth did not require any further destruction. Rapid scanning can be applied to a large number of samples to identify those which will most likely yield reasonable age estimations, leading to informed decisions about geochronological sampling strategies, including radiocarbon. We envisage that laser ablation spot analyses also could be used to obtain U-series depth profiles, which are required for open system dating, as well as depth profiles for other isotopes (e.g., Sr, Pb) to gain insights into prehistoric human migrations.

INTRODUCTION

U-series analysis is one of the few dating methods that can be applied for the direct dating of human fossils (e.g., Grün 2006). The newly developed laser ablation sampling technique only consumes minute amounts of material for mass spectrometric analysis (for the basis of the method see Eggins et al.[2003, 2005, for application on human materials, see Grün et al. 2005, 2006, 2008]). Nevertheless, the full application of U-series dating requires that a cross section of a bone is analyzed, so that diffusion processes can be assessed and samples unsuitable for U-series dating be rejected (e.g., Pike 2000, Pike et al. 2002). For sampling along a cross section, either by micro-drilling or by laser ablation, the specimen has to be cut. While the loss of mate-

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Figure 1. The two human teeth from Grotte de la Chèvre; CHE08-05, upper right first molar; CHE08-06, upper right third molar; from left to right: lingual, mesial, buccal, distal; views down: occlusal view, scale bar=1cm (pictures and artworks P-E Moullé and R. Colleter).

rial through the cut can be minimized using diamond wire saws, this sort of damage can be avoided for a significant number of specimens if it can be checked whether they are actually suitable for U-series analysis. Here we present a new strategy for rapid scanning of human teeth with laser ablation before detailed analysis is considered, which may involve the partial destruction of the specimen by cutting.

SAMPLES

The Grotte de la Chèvre is located in the Erve Valley of the Mayenne Department in Northwestern France. The cave is part of a system of around twenty caves and rock shelters currently identified in the valley. They are located in a carboniferous limestone overlying the crystalline and metamorphic rocks of the old Armorican basement. The Erve, which cut the valley from North to South, caused the formation of the small-sized karst. Some of the cavities are quite large, such as the Grotte Rochefort and Grotte Margot, which were occupied by humans during the Upper Paleolithic, Neolithic, and Bronze Age. The Grotte de la Chèvre is fronted by a double porch. It has a width of about fifteen meters. Its orientation to the south and the broad terrace in front of the entrances provide optimal conditions for human occupations during the Pleistocene and Holocene.

The site was excavated in the 19th century by Abbey Maillard and in the 1930s by Raoul Daniel. The collections from these excavations contain numerous artifacts from the Middle Paleolithic, Upper Paleolithic, and more recent times, including Mousterian sidescrapers and bifacial tools, Solutrean laurel leaves, Magdalenian harpoons, etc. Some of the faunal remains may even be older and were tentatively placed into the Middle Pleistocene, although human occupation of the cave at that time could be questioned (Hinguant et al. 2005). Unfortunately, no precise maps or reports were produced during these excavations, thus the precise position of any of the remains and their associations are actually unknown.

In 1999–2000 and then from 2007 to 2010, a series of surveys and excavations has been conducted in front of the porch and on the slope outside of the cave in order to assess the amount of archaeological *in situ* remains. This area was completely covered by the spoils of the nineteenth century excavations. There is no doubt that these sediments originated from the excavations of the deposits inside the cave, but any proveniencing is impossible. The collection of human remains contains 31 teeth, 10 cranial, and 65 postcranial remains, 61 were discovered during the recent excavations and 45 during the earlier. Based on metric and morphological comparisons, the dental remains should not be considered as a taxonomically homogenous group. Some teeth, such as CHE08-05 and CHE08-06, can be discriminated within the dental collection. The question is whether some of these specimens could be the oldest human fossils discovered in historic Brittany.

Two teeth were selected for U-series analysis. Sample CHE08-05 is an upper right 1st molar (Figure 1, left), while sample CHE08-06 is an upper right 3rd molar (Figure 1, right). Permission was granted to section the teeth to allow detailed U-isotope mapping, as it was carried out on the Neanderthal tooth from Payre (Grün et al. 2008). The elemental and isotopic maps of an area of the Payre tooth that was not covered by enamel showed that ^{232}Th was only found at the outside of the specimen and can be used to identify contamination with detrital Th derived from the sediment matrix. U-concentrations were highest close to the outside of the root. The ^{234}U/^{238}U activity ratios were very homogeneous throughout the mapped area. The highest ^{230}Th/^{234}U activity ratios and oldest ages were found on the surface of the root. These results served as a guide for the probing strategy of the Chèvre teeth. Instead of producing scans,

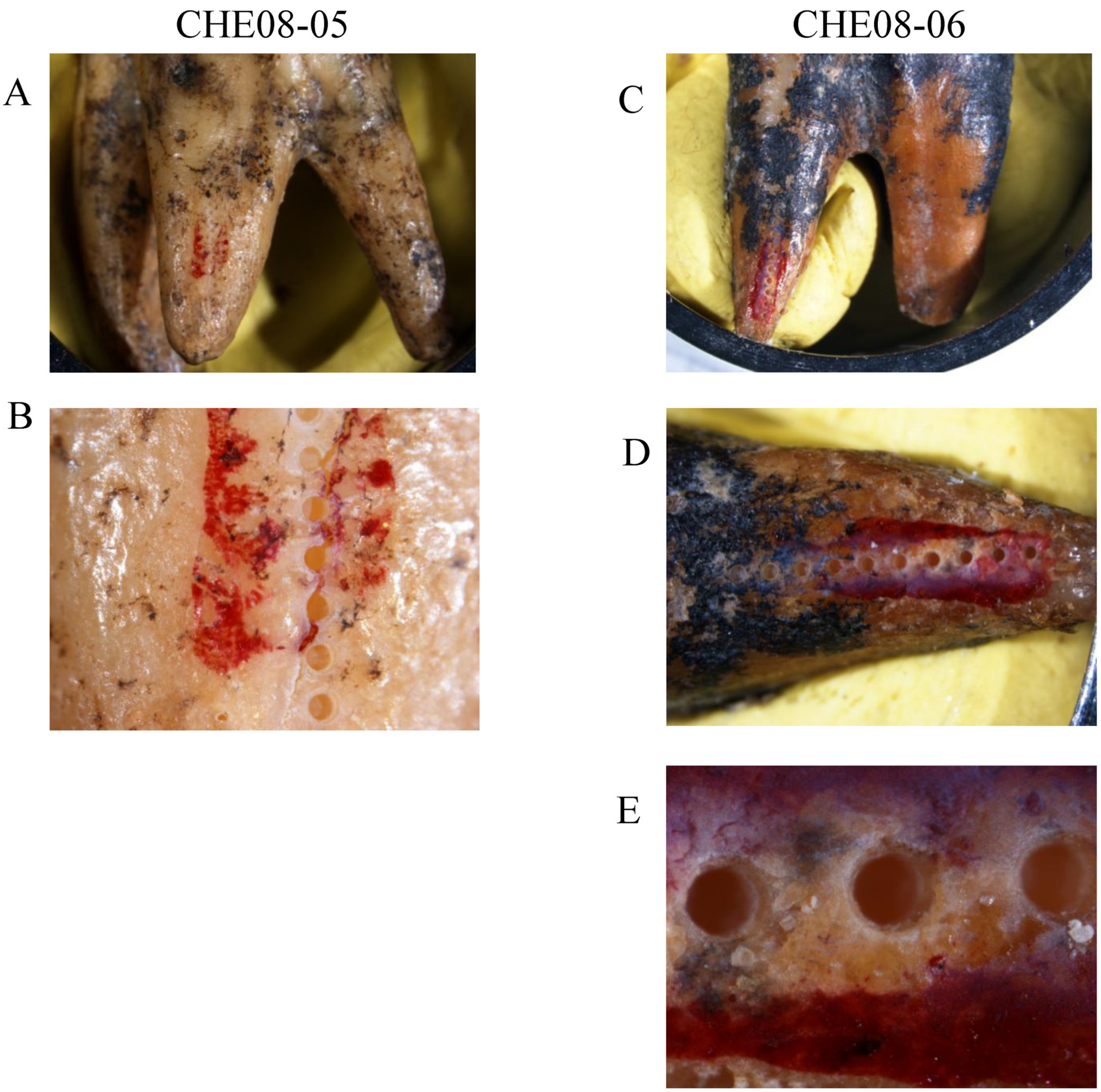

Figure 2. Photos of the two teeth after analysis. The red markers indicate the areas that are within the focal plane of the laser. First, a thin layer with a diameter of 233μm was ablated for cleaning, followed by the ablation for isotopic analysis using a spot size of 178μm. The cleaning step leaves a small rim (E).

the laser was used to drill holes. The ^{230}Th/^{238}U results could then be used to assess whether the specimen has sufficient amounts of ^{230}Th for more detailed age analysis.

EXPERIMENTAL

The analyses were carried out using a custom-built laser sampling system interfaced between an ArF Excimer laser (193 nm; Lambda Physik LPX120i) and a multi-collector Finnegan Neptune ICP mass-spectrometer. Details of this system and its capabilities were described previously (Eggins et al. 1998a, b). In brief, it employs a single long-working distance lens to project and demagnify (by a factor of 20) the image of a laser-illuminated aperture onto the sample surface, which enables a range of geometries to be ablated within bounding dimensions of between 1μm and 400μm.

The laser can only ablate within about one or two mm around its focal plane, which coincides with the upper rim of the steel holder. The teeth were mounted into the holders with yellow tack and then pressed down with a glass plate. The sections of the root that were approximately flush with the outer rim, i.e., lying in the focal plane of the laser, were colored with a red marker pen (Figure 2). This helps to find the respective area after the mount is transferred into the ablation cell. Both teeth were analyzed at ten sequential

TABLE 1. RESULTS OF THE LASER ABLATION SPOT ANALYSES (without elimination of spikes, see Figure 4). R08 = ^{230}Th/^{238}U activity ratio.

	CHE08-05		CHE08-06	
Spot	**R08**	**R08 error**	**R08**	**R08 error**
1	0.0181	0.1167	0.0114	0.0251
2	0.1292	0.4151	0.0046	0.0218
3	0.0693	0.2498	0.0154	0.0162
4	0.2460	0.4539	0.0150	0.0188
5	0.2692	0.2830	0.0149	0.0432
6	0.1744	0.3423	0.0460	0.0180
7	0.1507	0.3667	0.0894	0.0283
8	0.0710	0.1393	0.0181	0.0191
9	0.1661	0.3261	0.0294	0.0260
10	0.0429	0.0580	0.0251	0.0211

spots. To remove any surface contamination, each spot position was exposed to the laser set to a diameter of 233µm for 10s causing the removal of approximately 10µm of the sample. This was followed by the analytical run, where each position was ablated for 70s with the laser set to a diameter of 178µm. The wider rim of the cleaning run can be seen in Figure 2e. The total spot depth is around 100µm.

A drawback of the ANU system is that it has only one central ion counter, which has to be used for both ^{230}Th and ^{234}U analyses. For the calculation of a U-series age, ^{230}Th/^{238}U as well as ^{234}U/^{238}U ratios require measurement. This can only be done in consecutive measurements. Nevertheless, to assess the suitability of a sample for U-series dating it is only necessary to measure the ^{230}Th/^{238}U ratio. If there is insufficient ^{230}Th, no age can be calculated, if there is enough ^{230}Th for the estimation of a reasonable ^{230}Th/^{238}U ratio, there will be always enough ^{234}U to obtain a reasonable ^{234}U/^{238}U ratio in a subsequent analysis.

Data reduction followed established laser ablation ICP-MS protocols (after Longerich et al. 1996). For external calibration, tailing correction, and elemental fractionation, we used the international glass reference standard NIST SRM610 and a rhinoceros tooth from Hexian (sample 1118, see Grün et al. 1998), on which U-series ratios were established by repeated TIMS analysis. Mean background count rates measured with the 'laser off' were subtracted from all measured isotope intensities. All measured atomic ratios were converted into activity ratios.

RESULTS

The results of the spot analysis are shown in Figure 3 and Table 1. Note that the ^{232}Th is relatively amplified by a factor of 1000 compared to ^{238}U. The extremely low ^{232}Th voltages demonstrate that the cleaning run had successfully removed any detrital Th that may have been present at the surface of the teeth.

It is obvious that CHE08-05 contains very little uranium (see Figure 3, top). Both ^{230}Th and ^{232}Th are very close to the background, which was about 1.3 counts per second for the ^{230}Th measurement. The U-concentrations cannot be precisely measured because of the different peak shapes of the spots in the sample compared to the standard (compare the peaks of the samples in Figure 3 with those of the standard in Figure 5, below). Nevertheless, they are all well below 1ppm, except for spot #10. The ^{230}Th/^{238}U ratios are generally low, but have very large 2-σ errors of up to 400%. CHE08-05 contains significantly more U, in the range of 2 to 5ppm. Most ^{230}Th/^{238}U ratios have large errors, again up to 400%. Nevertheless, all ^{230}Th/^{238}U ratios are within errors <0.09 (spot #7). This would correspond to an age in the vicinity of 10 ka (using a minimum ^{234}U/^{238}U ratio of 1).

DISCUSSION

The surface analysis clearly showed that CHE08-05 was not suitable for laser ablation U-series dating at all. Thus, there is no need to cut this sample. Sample CHE08-06 contained significantly more uranium and yielded at least some ^{230}Th/^{238}U ratios at spots #6 and #7 that can be used for U-series dating. Considering that U-series analyses on bones usually provide minimum age estimates, the result initially implied that the tooth was older than perhaps 10 ka.

However, a closer examination of the ^{230}Th counts in the analysis of CHE08-06 shows occasional spikes in the ^{230}Th counts (circles in Figure 3, lower). For spot #7, most of the total count occurs in such a spot. These spikes are caused by small fragments entering the extraction line from the sample cell. These spikes do not occur during the background count that is carried out before the start of the ablation procedure and rarely after the measurement of the final NIST standard. This may point to an origin from the

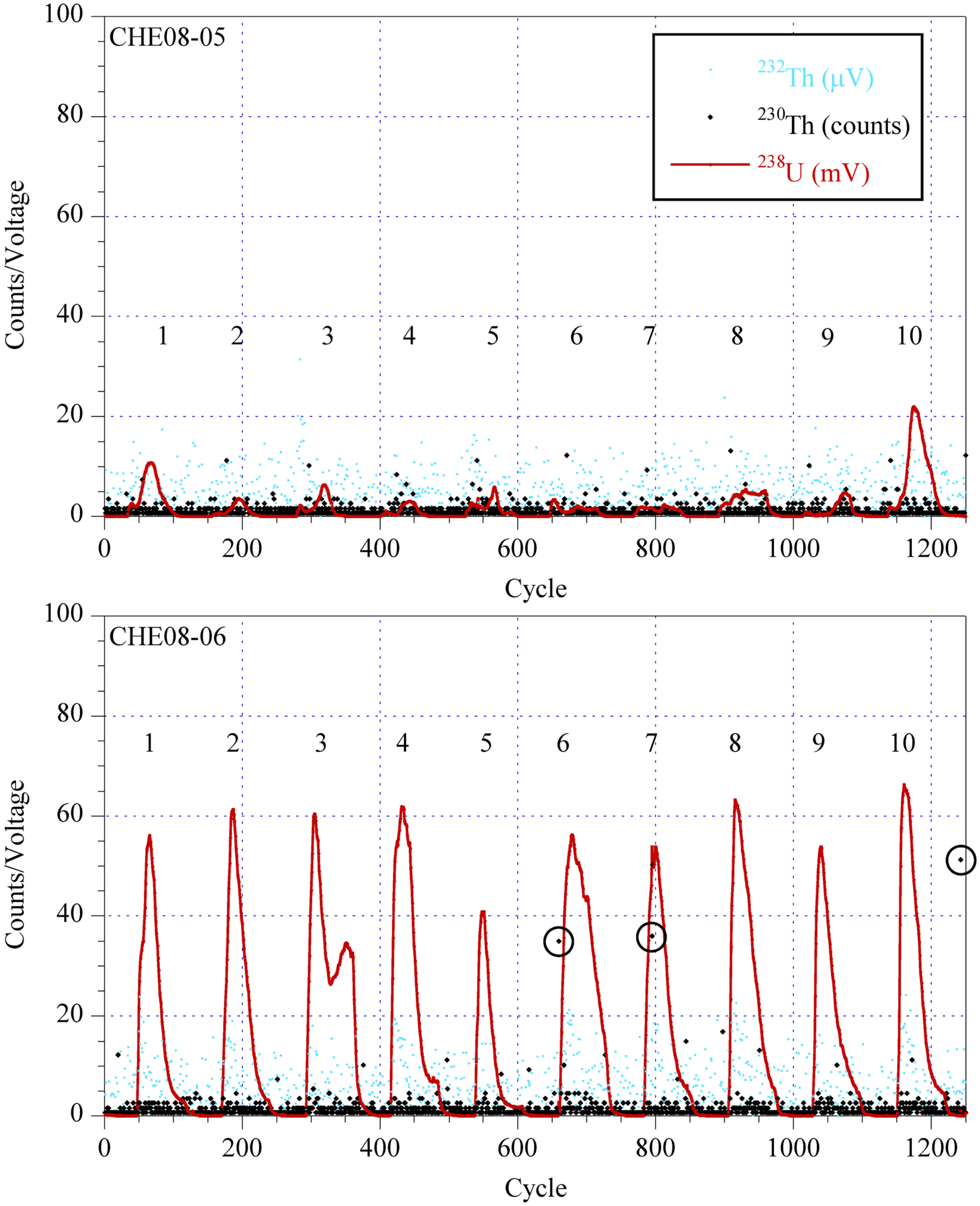

Figure 3. Charts of the isotope analyses. Each sample was analysed at ten positions. The circles in the lower diagram indicate spikes caused by small fragments reaching the plasma.

bone matrix, and small fragments are visible in Figure 2e around the ablation pits. When removing the obvious spike from the integrated ^{230}Th counts of spot #7, the total count is reduced by a factor of 8 (Figure 4). The other spots yielding somewhat larger ^{230}Th/^{238}U ratios, #6, 9 and 10, also show some ^{230}Th spikes. As a result, this sample yields preliminary ages in the range of a few thousand years at the most. While any U-series dating result should be regarded as minimum age, it is highly unlikely that the specimen could be many tens of thousands of years old. Thus, rather than being perhaps from the oldest human found in historic Brittany, the tooth most likely derives from an individual who lived in historic or perhaps late prehistoric times.

A question that remains is how far spot analysis can be pushed. In principle, it is possible to use the laser for drilling holes to depths between 1 and 2mm. The time series of U-series isotopes could provide the data for U-series age calculation according to the diffusion-adsorption model

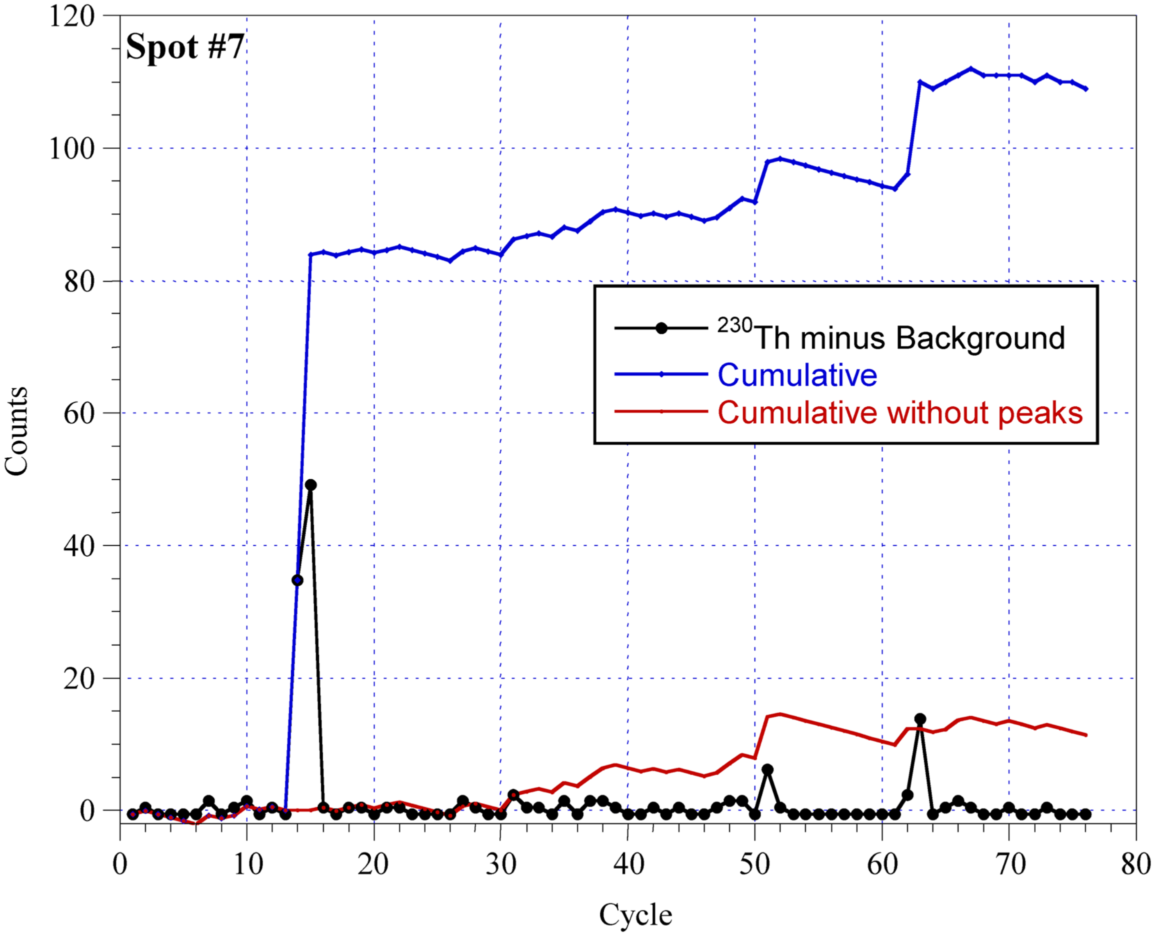

Figure 4. Details for Spot #7 of sample CHE08-06 (see Figure 3 lower). When eliminating the apparent spikes at cycles 14 and 15, 51 and 63, the cumulative counts are reduced by more than 80%.

(Millard 1993; Millard and Hedges 1996; Pike 2000; Pike et al. 2002). Ablating "deep" pits from the outside could alleviate the need for cutting samples altogether. Furthermore, U-series depth profiling could be used for the assessment of whether a bone or tooth is within the radiocarbon dating range. Though little carbon is required for the radiocarbon dating analysis itself, sample pretreatment techniques aimed at extracting the most pristine chemical compounds of older bones and teeth require significant amounts of raw sample, in the range of 500 to 1000mg (e.g., Higham et al. 2010). This would consume between a quarter to a half of the mass of the two teeth of this study.

Before applying this sampling strategy, however, there is at least one problem that has to be addressed, which relates to the behavior of Th in the ablation process. When comparing tracks with spots (Figure 5) there is a clear trend of increasing ^{230}Th counts with ablation depth, changing the resulting ^{230}Th/^{238}U ratio (see Figure 5, bottom). This does not apply to tracks (see Figure 5, top). At the moment, this effect can be explained by a process where after the atomization of the material by the laser, a small amount of Th is immediately plated out on the surface of the ablation pit. With increasing laser exposure, this deposited Th is re-ablated, thus Th becomes enriched during repeated cycles of deposition and re-ablation. In spot analysis, this problem is overcome by using a matrix matched standard and integration over the whole spot measurement. When drilling deeper holes, it is most likely that the recycling of Th may be highly matrix dependent, i.e., dense bones may behave quite differently from porous bones, resulting in strongly biased ^{230}Th/^{238}U depth profiles. This may perhaps be addressed by using a stepped ablation sequence in which subsequent pits are ablated with decreasing laser diameters.

Teeth are generally preferred in such analyses because bone fragments are often too large to be mounted in the ablation cell, i.e., for analysis, some sections would have to be cut off. The ablation characteristics of bones depend greatly on diagenesis, some bones are so soft that the ablation pits become very irregular. Furthermore, the bone matrix is heterogeneous and often contains many pores, which can be filled with contaminants (calcite, sediment etc.).

CONCLUSIONS

Our laser ablation system provides the opportunity to scan a large number of teeth for their suitability for U-series analysis. At the moment, perhaps 20 teeth (five spots each) can be scanned in a day and preliminary U-series age be

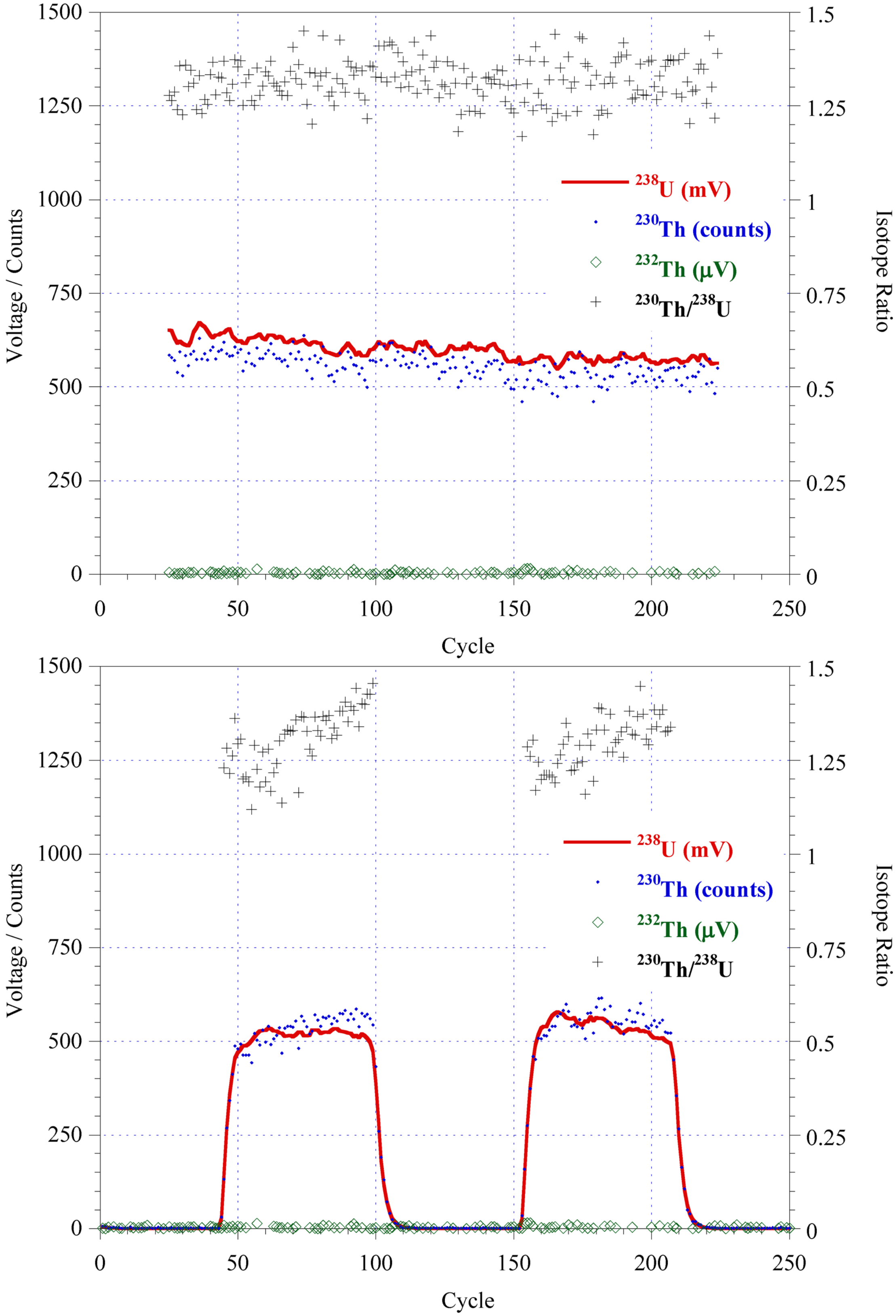

Figure 5. Differences between scan (top) and spot analysis (below) of the rhinoceros standard. The effective ionisation of ^{230}Th changes with depth in the ablation pit leading to biases in the calculation of Th/U isotope ratios.

calculated. This would provide a relatively low cost data set, from which detailed dating strategies can then be developed. In the immediate future, we will develop sampling procedures that allow the assessment of U-series isotope profiles along deep pits (up to 2mm). Along with U-series, other isotopes (e.g., Sr or Pb) sampled in this fashion could provide insights into human migrations. The actual amount of material required for such analyses is minute and any visible damage negligible.

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REFERENCES

Eggins, S., Grün, R., Pike, A.W.G., Shelley, A., and Taylor, L. 2003. ^{238}U, ^{232}Th profiling and U-series isotope analysis of fossil teeth by laser ablation-ICPMS. *Quaternary Science Reviews* 22, 1373–1382.

Eggins, S.M., Grün, R., McCulloch, M.T., Pike, A.W.G., Chappell, J., Kinsley, L., Shelley, M., Murray-Wallace, C.V., Spötl, C., and Taylor, L. 2005. In situ U-series dating by laser-ablation multi-collector ICPMS: new prospects for Quaternary geochronology. *Quaternary Science Reviews* 24, 2523–2538.

Eggins, S.M., Kinsley, L.K., and Shelley, J.M.G. 1998a. Deposition and element fractionation processes occurring during atmospheric pressure laser sampling for analysis by ICPMS. *Applied Surface Science* 127-129, 278–286.

Eggins, S.M., Rudnick, R.L., and McDonough, W.F.M. 1998b. The composition of peridotites and their minerals, a laser-ablation ICPMS study. *Earth and Planetary Science Letters* 154, 53–71.

Higham, T., Jacobi, R., Julien, M., David, F., Basell, L., Wood, R., Davies, W., and Ramsey, C.B. 2010. Chronology of the Grotte du Renne (France) and implications for the context of ornaments and human remains within the Chatelperronian. *Proceedings of the National Academy of Sciences of the United States of America* 107, 20234–20239.

Grün, R. 2006. Direct dating of human remains. *Yearbook of Physical Anthropology* 49, 2–48.

Grün, R., Aubert, M., Joannes-Boyau, R., and Moncel, M.H. 2008. High resolution analysis of uranium and thorium concentrations as well as U-series isotope distributions in a Neanderthal tooth from Payre using laser ablation ICP-MS. *Geochimica Cosmochimica Acta* 72, 5278–5290.

Grün, R., Huang, P.H., Huang, W., McDermott, F., Stringer, C.B., Thorne, A., and Yan, G. 1998. ESR and U-series analyses of teeth from the palaeoanthropological site of Hexian, Anhui Province, China. *Journal of Human Evolution* 34, 555–564.

Grün, R., Maroto, J., Eggins, S., Stringer, C., Robertson, S., Taylor, L., Mortimer, G., and McCulloch, M. 2006. ESR and U-series analyses of enamel and dentine fragments of the Banyoles mandible. *Journal of Human Evolution* 50, 347–358.

Grün, R., Stringer, C., McDermott, F., Nathan, R., Porat, N., Robertson, S., Taylor, L., Mortimer, G., Eggins, S., and McCulloch, M. 2005. U-series and ESR analyses of bones and teeth relating to the human burials from Skhul. *Journal of Human Evolution* 49, 316–334.

Hinguant, S., Moullé, P.E., and Arellano, A. 2005. Premiers indices de la présence d'une faune du Pléistocène moyen dans la vallée de l'Erve. *Bulletin du Musée d'Anthropologie Préhistorique de Monaco* 45, 25–30.

Longerich, H.P., Jackson, S.E., and Gunther, D. 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. *Journal of Analytical Atomic Spectrometry* 11, 899–904.

Millard, A.R. 1993. *Diagenesis of archaeological bone: the case of uranium uptake*. D.Phil. Thesis, University of Oxford.

Millard, A.R. and Hedges, R.E.M. 1996. A diffusion-adsorption model of uranium uptake by archaeological bone. *Geochimica et Cosmochimica Acta* 60, 2139–2152.

Pike, A.W.G. 2000. *Uranium series dating of archaeological bone by thermal ionization mass spectrometry*. D.Phil. Thesis, University of Oxford, Oxford.

Pike, A.W.G., Hedges, R.E.M., and Van Calsteren, P. 2002. U-series dating of bone using the diffusion-adsorption model. *Geochimica et Cosmochimica Acta* 66, 4273–4286.