



# The use of laser spectroscopy to investigate bone disease in King Henry VIII's sailors



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## ABSTRACT

The *Mary Rose* was King Henry VIII's flagship before it sank in battle on the 19th July 1545. Over four hundred men went down with the ship and the environment of the Solent meant their remains were quickly covered in silt. Between 1979 and 1982 the remains of 179 individuals were recovered and examined as part of the excavation of the *Mary Rose*. The anaerobic environment created by the silt preserved the sailors' bones in remarkable condition and to date much has been learnt about life on the ship. In this study we used Raman spectroscopy (a non-destructive technique), to investigate the chemistry of the human bones, specifically for the identification of disease in archaeological specimens, for the first time. Raman data were collected from five anatomically normal tibiae and five tibiae that were bowed (individuals suspected to have suffered from bone disease in childhood). The data were processed using multivariate analysis (principal component analysis) and results showed the presence of chemical abnormalities in the bowed bones which resulted in the separation of the bones into two clearly defined groups, normal and bowed.

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## 1. Introduction

The *Mary Rose*, King Henry VIII's flagship, was built in Portsmouth in 1509–10 and was in service until she sank just outside Portsmouth Harbour during the Battle of the Solent on the 19th July 1545 (Rule, 1982; Stirland, 2000). The crew of over 400 men were trapped by the anti-boarding netting fitted above the upper deck and the vast majority drowned (only 35–40 survived). The geography of the Solent creates a unique tidal regime which deposited soft sediments on the wreck of the *Mary Rose*. The layers of sediments created anaerobic conditions, which preserved much of the organic material, including the human bones, exceptionally well (Stirland, 2000).

*Abbreviations:* PCA, principal component analysis.

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A major excavation of the *Mary Rose* between 1979 and 1982 yielded more than 25,000 objects, including a large number of human bones. Although the soft tissue no longer remained, the bones were preserved, however no completely articulated skeletons were found. Although many areas contained dense accumulations of bones from a number of individuals, post excavation sorting revealed an assemblage of at least 179 individuals, 92 of which have been partially reconstructed based on bone morphology and archaeological association. All individuals on board were men with an estimated biological age range of 12–40 years, but the majority was aged 18–30 years. There is no crew list from the ship and only three individuals are known by name, unfortunately none of these can be identified from the assemblage recovered. As re-constructing individuals required fitting the bones together, specific bones such as shoulders, hands, feet, ribs and breastbones could not be included unless the archaeological association was overwhelming. Similarly, unless most of the spinal column was present, skulls were not assigned to individuals (Stirland, 2005).

Of the 1241 long limb bones that were excavated from the wreck, two femora out of 319 were significantly bowed

anteroposteriorly, and 12 tibiae out of 310 showed significant bowing mediolaterally, suggestive of residual rickets (Roberts, 2008; Brickley and Ives, 2008). Rickets is a childhood disease resulting from a lack of dietary phosphate and/or calcium and reduced sun exposure. The skeletal manifestation is bending and flaring of the bone: active rickets. Once the deficiency is treated as the bone turns over and remodels a full recovery is usually expected (healed rickets). However, when this does not occur individuals may have residual rickets. For definitive diagnosis of rickets (active and residual) in archaeological skeletal remains, evidence would also need to be confirmed in the skull and ribs; although radiological evidence of thickening of the concaved cortex with a shaped cortical outline would reduce the likelihood of a differential diagnosis (Brickley and Ives, 2008; Mays et al., 2006). In addition to bowing and swelling, increased porosity would also be evident in active rickets (Mays et al., 2006). To note, the majority of diagnosed rickets from bending deformities in archaeological adult specimens are cases of residual rickets (Mays et al., 2006). In the majority of cases, from the bones retrieved from the *Mary Rose*, the three types of specimen were not present in a single reconstructed individual. The other possible conditions that are associated with “long bone bending deformities and metaphyseal swelling” are osteogenesis imperfecta, metaphyseal chondrodysplasia and Blount’s disease (Brickley and Ives, 2008). These disorders are either very rare and result in multiple fractures and/or short stature (osteogenesis imperfecta and metaphyseal chondrodysplasia) or they progress in such a way as to leave individuals severely disabled by military age (Blount’s disease). There was no evidence of multiple fractures or that the bones were from individuals of short-stature: tibiae with fused epiphyses were 25–30 cm in length. Although the bones have bending deformities, there was no evidence that they were severe and therefore unlikely to result in disability.

Raman spectroscopy is a non-destructive technique, in which laser light of a single wavelength is scattered from the surface of an object, collected, and analysed. The scattered light contains wavelength-shifted components and this spectrally shifted light, when plotted as a spectrum (magnitude of shift versus intensity of scattered light), represents a unique chemical ‘fingerprint’ for a particular substance. For simple substances with a small number of chemical bonds the Raman spectra may be easy to elucidate but for complex molecules and mixtures (e.g. biological samples) and for cases where differences are subtle, more advanced analysis techniques are required. Commonly, principal component analysis (PCA) is used to reduce the number of variables by forming linear combinations of the wavenumbers and ranking them by degree of variance. The data may then be plotted in scatter plots along these new axes to look at how the data are grouped or spread. The inspection of these new axes may facilitate the identification of the spectral region, and therefore the biochemical(s), which are responsible for the grouping or spreading of the data. Further analyses can also be performed on the PCA-transformed data set.

Raman spectroscopy as a non-destructive technique represents an excellent method for characterising the chemical nature of valuable archaeological samples and has been applied to artworks and artefacts, (Smith and Clark, 2004) fossilised charcoal from archaeological sites, (Cohen-Ofri et al., 2006) and Roman age pottery, stone and glass (Ricciardi et al., 2009). Raman spectroscopy has also been applied to the study of ancient and fossilized teeth with two studies showing that the carbonate and collagen content of teeth decreases with time, and another to study pigmentation of human remains (Carden and Morris, 2000).

The dental studies illustrate an analytical strength of Raman spectroscopy: it can be used to probe both the mineral and organic phases of mineralised tissues (in contrast to X-ray techniques which probe the mineral phase). In recent years Raman

spectroscopic studies of bone diseases in the medical and biomedical fields have led to new insights in the etiology/diagnosis of osteoarthritis, osteoporosis and osteogenesis imperfecta (Morris, 2010).

The purpose of the present study is to use (non-destructive) Raman spectroscopy and multivariate analysis to obtain chemical information from the protein and mineral components of the human bones that have spent 437 years under the sea. Specifically, we aim to compare bowed archaeological specimens with anatomically normal control bones to determine if there are chemical modifications present which may enhance our understanding of the presence of metabolic bone disease in 16th century human populations.

## 2. Materials and methods

### 2.1. Specimens

Ten tibiae from adult men were obtained from the *Mary Rose* Trust in Portsmouth, UK (See Suppl. Table 1 for details). Five of the tibia had a normal anatomical appearance (Fig. 1, top) and five showed signs of significant proximal mediolateral bending suggestive of one of “various metabolic bone diseases in which bone shape can be altered by mechanical loading in response to an underlying pathological condition” (Fig. 1, middle) (Brickley and Ives, 2008). Bones with evidence of multiple pathologies, including osteoarthritis, were excluded from this study.

A fresh cadaveric human tibia from an adult male was also obtained (from Vesalius Clinical Training Centre, University of Bristol; ethics approval no. 08/H0724/34), so the spectra could be compared. That tibia was from a 58 year old individual who had died due to a soft-tissue cancer (the bone was examined by an orthopaedic surgeon and showed no signs of bone disease). The cadaver was frozen on being donated (193 K) and after being excised the tibia was stored at 193 K. All the soft tissue associated with the tibia was removed with a scalpel before it was scanned.

### 2.2. Raman spectroscopy

The Raman spectra were collected with an instrument that was custom built (Cobalt Light Systems Ltd., Oxfordshire, UK). The instrument utilised an 830 nm laser (300 mW at sample), a low loss Opttran WF fibre-optic bundle (CeramOptec, MA, USA) and a Raman

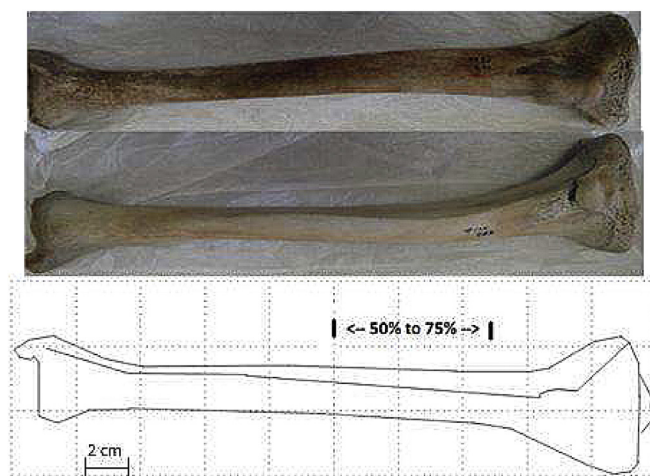


Fig. 1. Top – normal tibia bone from the *Mary Rose*; middle – bowed tibia bone from the *Mary Rose*; bottom – outline showing where Raman data was collected.

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