Raman Spectroscopy in Forensic Science and Anthropology: Current Applications and Emerging Perspectives

Fayçal Dergal*

Abstract— **Forensic anthropology faces an enduring methodological tension: the imperative to extract maximum information from skeletal and dental remains while preserving their evidentiary integrity for downstream analyses such as DNA profiling, stable-isotope quantification, or radiocarbon dating. Raman spectroscopy a vibrational technique that probes the molecular fingerprint of a sample through inelastic light scattering has emerged as a compelling response to this constraint. Operating without physical contact, chemical reagents, or destructive sample preparation, the technique yields richly detailed spectra that encode both organic (collagen, lipids, proteins) and inorganic (hydroxyapatite, carbonates) constituents of mineralized tissues. Over the past decade, independent research groups have demonstrated that Raman-based measurements can contribute meaningfully to several forensic objectives: estimating the post-mortem interval from the progressive diagenetic alteration of bone, discriminating human from non-human osseous material, assessing the thermal history of burned or cremated remains, determining biological age and sex from dental hard tissues, and pre-screening specimens for DNA viability prior to costly extraction protocols. Portable and handheld spectrometers now bring these capabilities directly to the field, opening a realistic prospect of on-site triage at crime scenes and disaster contexts. The present mini-review provides a critical synthesis of the published literature on these applications, identifies the principal analytical challenges fluorescence interference, limited spatial resolution, the need for robust chemometric pipelines and outlines the directions along which the technique is most likely to mature in the coming years. To situate the technique within the wider analytical landscape, an explicit comparison is drawn with FTIR spectroscopy, X-ray diffraction, SEM-EDS, mass spectrometry, DNA typing and stable-isotope analysis. The chemometric and machine-learning pipelines (principal component analysis, PLS discriminant analysis and regression, random forests, support-vector machines and convolutional neural networks) upon which the quantitative interpretation of forensic Raman data increasingly depends are examined in turn, together with the validation requirements they impose. Throughout, a deliberate distinction is maintained between demonstrated analytical capability and validated routine forensic application: for most of the applications surveyed here the published evidence remains at the proof-of-concept or early-validation stage.**

Keywords— **Forensic anthropology; non-destructive analysis; post-mortem interval; Raman spectroscopy; SERS; skeletal remain; chemometrics; machine learning; FTIR; X-ray diffraction; forensic method validation.**

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F. Dergal is with the Centre for Scientific and Technical Research on Physicochemical Analysis (CRAPC), Tipaza/Algeria (email : dergalf@yahoo.fr)

I. INTRODUCTION

Identifying and characterizing human remains constitutes one of the oldest yet persistently challenging mandates in forensic science. Whether the context involves criminal investigations, mass-disaster victim identification, or the repatriation of war dead, practitioners routinely confront specimens in advanced states of decomposition, skeletonization, or thermal alteration that render classical morphological assessment ambiguous at best [1,2]. Against this backdrop, the forensic community has progressively embraced instrumental analytical techniques capable of extracting molecular-level information that macroscopic examination alone cannot furnish.

Among vibrational spectroscopic methods, Raman spectroscopy occupies a distinctive niche. First described by C.V. Raman in 1928, the phenomenon relies on the inelastic scattering of monochromatic light by molecular vibrations, generating a spectrum that serves as a true molecular fingerprint [3]. Bone, teeth, hair, and nails the tissues most frequently encountered by forensic anthropologists are, from a physicochemical standpoint, composite materials whose organic and inorganic phases each produce well-resolved Raman signatures. The intense symmetric stretching mode of phosphate (ν_1 PO_4^{3-}) near 960 cm-1 tracks the crystallinity and stoichiometry of bioapatite, while the amide I (~1665 cm^{-1}), amide III (~1245 cm^{-1}), and CH_2 wag (~1450 cm-1) bands report on the state of collagen and other proteinaceous matrix components [4,5]. This dual sensitivity is what makes the technique so well suited to forensic inquiries that hinge on the interplay between mineral and organic phases.

Equally significant from a forensic standpoint is the non-destructive character of Raman analysis. Unlike mass spectrometry, chromatographic assays, or even attenuated total reflectance infrared spectroscopy (ATR-FTIR), a Raman measurement deposits negligible energy on the specimen, leaves no physical imprint, and requires no consumable reagents [6]. Specimens can therefore be directed without delay to subsequent examinations DNA extraction, radiocarbon dating, stable-isotope profiling without any loss of material or alteration of its chemistry. The practical relevance of this attribute cannot be overstated in medico-legal contexts, where the chain of custody demands that evidence remain as intact as possible.

Miniaturization of Raman instrumentation has added a further dimension. Handheld and portable spectrometers, now commercially available from several manufacturers, deliver analytical performance that begins to rival benchtop systems, with acquisition times on the order of seconds [7]. The prospect of performing a first chemical screening directly at a burial site, a fire scene, or a clandestine grave before any specimen is bagged and transported has motivated a growing body of applied research that this review seeks to critically evaluate.

In the present contribution, the principal forensic-anthropological domains are surveyed in which Raman

spectroscopy has demonstrated tangible utility: post-mortem interval estimation, species discrimination, thermal-history reconstruction, biological profiling from dental tissues, assessment of diagenetic alteration, and body-fluid detection. For each domain, the underlying spectroscopic rationale is outlined, the most salient empirical findings are discussed, and the remaining analytical gaps are identified. A concluding section considers the trajectory of the field and the role that emerging modalities spatially offset Raman spectroscopy (SORS), surface-enhanced Raman spectroscopy (SERS), and Raman hyperspectral imaging may come to play. Because several of the modalities surveyed here, in particular surface-enhanced Raman spectroscopy applied to biological traces, operate at the interface between forensic anthropology sensu stricto and forensic science more broadly, the scope of the present review has been framed accordingly. Skeletal and dental evidence is treated as the core subject matter, while the associated biological traces recovered from the same medico-legal context are considered wherever they bear directly on the recovery, interpretation, or identification of human remains.

II. PHYSICOCHEMICAL BASIS OF RAMAN ANALYSIS OF MINERALIZED TISSUES

Bone is, at the most fundamental level, a nanocomposite: carbonated hydroxyapatite crystallites, approximately 50 nm in length, are deposited along and between tropocollagen fibrils whose triple-helix architecture confers tensile resilience [4]. The Raman spectrum of fresh cortical bone accordingly presents a superposition of mineral and organic features. The phosphate ν_1 band near 960 cm^{-1} dominates the spectrum and is sensitive to crystallite size, carbonate substitution, and lattice strain. The carbonate ν_1 band near 1070 cm^{-1} quantifies type-B carbonate substitution in the apatite lattice, while the ratio of these two features commonly termed the mineral-to-matrix ratio or the carbonate-to-phosphate ratio has been adopted as a proxy for bone maturation, diagenetic alteration, and pathological mineralization [5].

As regards the organic matrix, it is probed primarily through the amide **I** band (~1665 cm^{-1}, arising from C=O stretching of the peptide backbone), the amide III band (~1245 cm^{-1}, a mixed mode involving C–N stretching and N–H bending), and the proline/hydroxyproline ring vibration near 855 cm^{-1} [4]. A ratio of the 960 cm^{-1} and 1636 cm^{-1} peak heights, as demonstrated by France and al. [8], provides the most unambiguous distinction between bones with well-preserved and poorly-preserved collagen a parameter of immediate relevance to isotopic and genetic analyses that require intact protein or DNA.

Dental enamel and dentin, though compositionally related to bone, differ in their organic-to-mineral proportion. Enamel, the hardest tissue in the human body, is approximately 96% mineral by weight, producing Raman spectra overwhelmingly dominated by the phosphate modes. Dentin, richer in collagen (~20% by weight), yields spectra more closely resembling those of bone [9]. These differences have direct forensic implications: enamel spectra are optimal for crystallographic analyses (e.g., carbonate content, crystallinity index), whereas dentin spectra are better suited to age-related changes in the organic matrix.

For ease of reference, the vibrational assignments that underpin the forensic interpretation of Raman spectra of mineralized tissues are collected in Table 1, and a representative spectrum of human cortical bone is reproduced in Figure 1. Three features merit emphasis. First, the ν1 PO_4^{3-} band near 960 cm^{-1} dominates the fingerprint region, typically exceeding the amide I intensity by a factor of five to ten in fresh cortical bone. Second, its full width at half maximum (FWHM), of the order of 15 to 20 cm^{-1} in unaltered bone and falling below 10 cm^{-1} in specimens heated above 600 °C, provides a direct index of apatite crystallite size and lattice order. Third, virtually every forensic parameter derived from these spectra is expressed as a band-area or band-height ratio rather than as an absolute intensity, which confers a welcome insensitivity to laser power, focal depth and surface roughness, precisely the three variables that are least well controlled under field conditions [10].

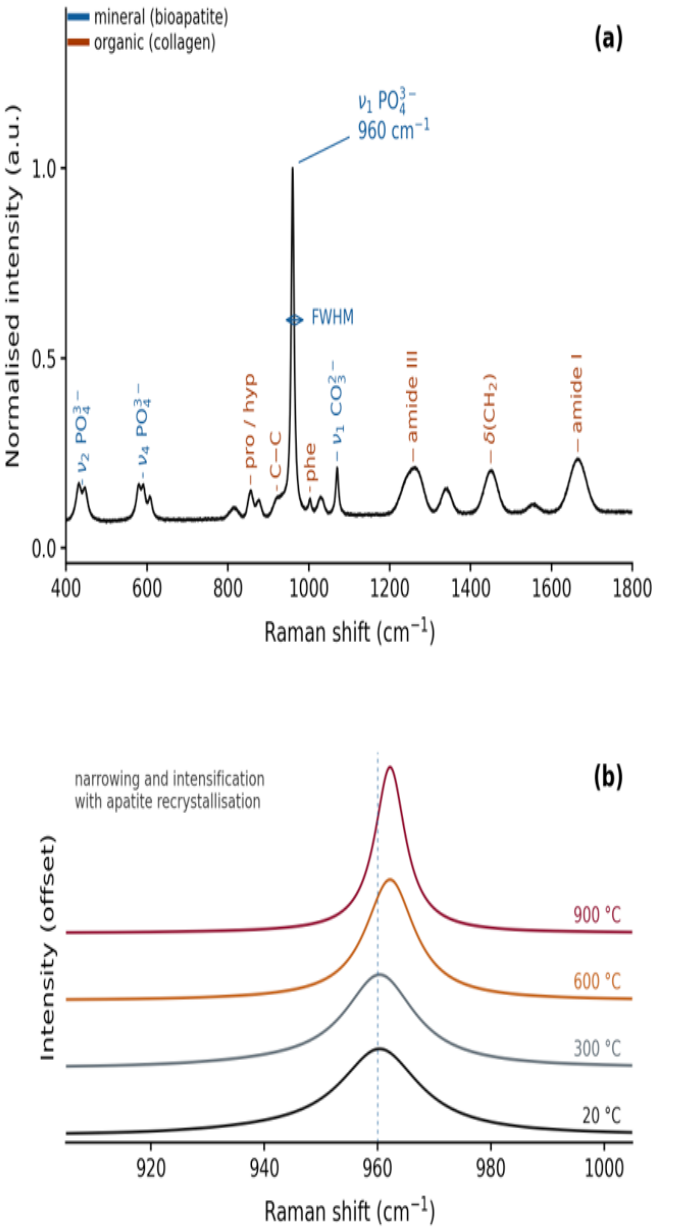


Fig. 1. Principal Raman features of mineralized tissue. (a) Representative fingerprint-region spectrum of human cortical bone showing the mineral (blue) and organic (red) band assignments listed in Table I. (b) Evolution of the ν_1 PO_4^{3-} band with increasing thermal exposure: progressive narrowing and intensification accompany the recrystallization of bioapatite above approximately 600 °C. The traces have been reconstructed from band positions, widths and relative intensities reported in the literature [4,5,10,11,12] and are intended as a didactic reference rather than as experimental data.

Table I.

PRINCIPAL RAMAN BANDS OF MINERALIZED TISSUES AND THEIR FORENSIC SIGNIFICANCE.

Raman shift (cm^{-1})	Vibrational assignment	Phase	Forensic significance
430-450	ν_2 PO_4^{3-} bending	Mineral	Lattice order; used with ν_4 to follow apatite maturation
570-615	ν_4 PO_4^{3-} bending	Mineral	Ancillary crystallinity marker; well resolved in enamel
815	C–C stretching (collagen backbone)	Organic	Weak band; declines with hydrolytic degradation of collagen
856 / 876	Proline / hydroxyproline ring	Organic	Collagen-specific; primary marker of protein preservation
920–940	C–C stretching; non-apatitic phosphate	Mixed	Shoulder tracking disordered mineral environments
960	ν_1 PO_4^{3-} symmetric stretching	Mineral	Dominant band; position, intensity and FWHM give mineral content and crystallinity
1003	Phenylalanine ring breathing	Organic	Internal reference for protein content
1030-1048	ν_3 PO_4^{3-} antisymmetric stretching	Mineral	Sensitive to carbonate substitution in the apatite lattice
1070	ν_1 CO_3^{2-} (type-B carbonate)	Mineral	Carbonate-to-phosphate ratio; diagenesis and thermal alteration
1245-1275	Amide III (C–N stretch, N–H bend)	Organic	Collagen secondary structure; loss indicates denaturation
1450	$\delta(CH_2)$ deformation	Organic	Total organic content; used in mineral-to-matrix ratios
1660-1670	Amide I (C=O stretching)	Organic	Most widely used organic marker; mineral-to-matrix ratio
2940	C–H stretching	Organic	Lipid and protein contribution in decomposed remains

Derived parameters most frequently reported in forensic studies: mineral-to-matrix ratio (ν_1 PO_4^{3-}/amide I, or ν_1 PO_4^{3-}/$\delta(CH_2)$); carbonate-to-phosphate ratio (ν_1 CO_3^{2-}/ν_1 PO_4^{3-}); crystallinity index (inverse FWHM of ν_1 PO_4^{3-}); collagen quality ratio (I_{960}/I_{1636}). Band positions vary by a few wavenumbers with excitation wavelength, tissue type and degree of diagenetic alteration.

III. POSITIONING RAMAN SPECTROSCOPY WITHIN THE FORENSIC ANALYTICAL WORKFLOW

Raman spectroscopy is rarely, and should rarely be, deployed in isolation. Its practical value in a medico-legal investigation depends on where it is inserted in a sequence of examinations that typically begins with macroscopic and radiographic assessment and may end with DNA typing or isotope-ratio mass spectrometry. Because each of these techniques answers a different question, at a different cost in time, expertise and irreplaceable material, an explicit comparison is warranted; Table II summarizes the principal contrasts [1,10].

The closest analytical relative of Raman spectroscopy is infrared absorption spectroscopy, and the two are best regarded as complementary rather than competing. The selection rules differ: Raman scattering responds to changes in molecular polarizability and is therefore most sensitive to symmetric, non-polar vibrations, whereas infrared absorption responds to changes in dipole moment and favours polar bonds. In practice the ν_1 PO_4^{3-} mode, weak and poorly resolved in FTIR, is the dominant feature of the bone Raman spectrum, while the ν_3 PO_4^{3-} envelope and the carbonate modes used to compute the infrared crystallinity and carbonate indices are better resolved by FTIR [13]. The decisive operational difference nevertheless lies elsewhere: water is a strong infrared absorber but a weak Raman scatterer, so damp or freshly exhumed specimens can be interrogated by Raman spectroscopy with no drying step, whereas ATR-FTIR ordinarily requires desiccation and, in its most common implementation, abrasion of a small quantity of powder from the specimen. Studies applying both techniques to the same skeletal series report broadly concordant diagenetic trends, with FTIR offering superior signal-to-noise on the mineral phase and Raman offering non-contact acquisition together with richer information on the organic matrix [14].

X-ray diffraction addresses a different level of description altogether. Where Raman spectroscopy reports on short-range vibrational order, XRD measures long-range crystallographic periodicity and yields unit-cell parameters and coherent domain sizes that vibrational methods can only approximate [15]. For the reconstruction of thermal histories, in which the transformation of poorly crystalline bioapatite into a sintered, well-ordered apatite is the phenomenon of interest, the two approaches are highly complementary, and the studies that have combined them provide the most robust temperature calibrations currently available [11]. XRD nevertheless requires a powdered or at least flat and homogeneous specimen, and it is silent on the organic phase. SEM-EDS, for its part, delivers elemental composition and microstructural imaging at high spatial resolution, but under vacuum and usually with a conductive coating, and it is blind to molecular speciation: it can establish that calcium and phosphorus are present in a given ratio, but it cannot distinguish carbonated bioapatite from a secondary diagenetic calcium phosphate.

The mass-spectrometric, genetic and isotopic methods occupy the opposite end of the spectrum in specificity and in cost. Peptide mass fingerprinting of bone collagen discriminates species with a taxonomic resolution that vibrational spectroscopy cannot approach [16], and STR or mitochondrial typing provides individual identification; but all of these consume material, require laboratory infrastructure, and fail unpredictably on poorly preserved specimens [17]. It is precisely this unpredictability that defines the most defensible current role for Raman spectroscopy in the forensic workflow: not as a substitute for these methods, but as a rapid, non-consumptive triage stage placed upstream of them, capable of ranking specimens by the probability that a destructive analysis will succeed [18,19]. Reviews of non-destructive Raman methodology developed for cultural-heritage materials, where the requirement of absolute specimen preservation is at least as stringent as in forensic practice, describe acquisition protocols and chemometric strategies that transfer directly to this triage role [20].

Read as a whole, Table II suggests a straightforward operational principle: Raman spectroscopy should be applied first, because it costs nothing in material and forecloses no subsequent examination, and the techniques that consume the specimen should be applied last, selectively, and on the basis of the information that the non-destructive stage has already provided.

Table II.

COMPARATIVE POSITIONING OF RAMAN SPECTROSCOPY AND THE PRINCIPAL COMPLEMENTARY TECHNIQUES USED IN THE FORENSIC ANALYSIS OF SKELETAL AND DENTAL REMAINS.

Technique	Information obtained	Sample consumption and preparation	Role in the forensic workflow, and principal limitation
Raman spectroscopy (including SORS and SERS)	Molecular composition of the mineral and organic phases; crystallinity; state of collagen	None; contactless and non-consumptive. Specimen analysed as recovered, wet or dry	Rapid triage and screening; PMI, thermal-history and species indicators; on-scene deployment. Limited by fluorescence; yields no elemental or long-range structural information
ATR-FTIR spectroscopy	Mineral and organic composition; infrared crystallinity and carbonate indices	Low; drying required, and usually micro-sampling or surface abrasion	Established laboratory assessment of bone diagenesis. Requires physical contact; water absorbs strongly; weak ν_1 PO_4^{3-} response
X-ray diffraction	Long-range crystal structure; unit-cell parameters; crystallite size	Low to moderate; powdering and flat mounting usual	Reference method for heat-induced recrystallization and thermal calibration. Silent on the organic phase
SEM-EDS	Elemental composition; surface and internal microstructure	Low; sub-sampling, vacuum-compatible mounting and conductive coating	Microstructural and elemental characterization; diagenetic inclusions. No molecular speciation; light elements poorly quantified
Mass spectrometry (peptide mass fingerprinting, proteomics)	Peptide and protein sequences; taxonomic assignment	Destructive, typically 10–50 mg; demineralization and enzymatic digestion	Definitive species identification where vibrational methods are inconclusive. Consumes material; depends on collagen survival
DNA analysis (STR, mtDNA)	Individual identification; sex; kinship	Destructive, typically 0.2–1 g; cleaning, powdering and extraction	Definitive individual identification. Expensive; fails frequently on degraded specimens
Stable-isotope analysis (IRMS)	Diet, geographic origin,, and mobility	Destructive, typically 10–500 mg; collagen or bioapatite extraction	Provenancing and biological profiling. Requires well-preserved collagen; provides no information on identity

Figures indicate typical practice reported in the forensic and archaeometric literature; they vary with instrumentation, specimen condition and laboratory protocol.

IV. POST-MORTEM INTERVAL ESTIMATION FROM SKELETAL REMAINS

Estimating the time elapsed since death the post-mortem interval (PMI) is among the most pressing and most contentious questions in forensic practice. For fleshed remains, entomological and biochemical indicators provide reasonable estimates within the first weeks. Once skeletonization is complete, however, available methods become sparse, and their accuracy diminishes steeply [1,2]. Raman spectroscopy has been explored as a means of filling this analytical void by tracking the progressive chemical modification that bone undergoes during burial.

At the mechanistic level, the rationale is anchored in the phenomenon of diagenesis: upon interment, bone begins to exchange ions with the surrounding soil pore water, collagen undergoes hydrolytic and microbial degradation, and the crystallinity of the apatite lattice increases as smaller, less-ordered crystallites dissolve preferentially and reprecipitate onto larger, more thermodynamically stable ones [21,22]. Each of these processes leaves a spectroscopic signature. McLaughlin and Lednev [23] showed that the mineral-to-matrix ratio, the carbonate-to-phosphate ratio, and the full width at half maximum (FWHM) of the ν_1 PO_4^{3-} band all correlate with burial duration in controlled taphonomic experiments spanning intervals from days to several years.

A particularly compelling study by Woess *et al.* [24] undertook a scoping review of the literature on Raman-based PMI estimation and concluded that, while the technique holds genuine promise, a number of confounding variables soil pH, temperature regime, moisture content, microbial load, and the anatomical element sampled have yet to be adequately controlled. The practical consequence is that a single Raman spectrum cannot, at present, yield a reliable standalone PMI; the data must be interpreted within the framework of site-specific taphonomic context. Building on this observation, recent work by Baptista *et al.* [14] has integrated Raman spectral data with multivariate chemometric models (principal component analysis and partial least-squares regression) to improve predictive accuracy, achieving errors of roughly ±2 years for burials in the 0–30 years range.

A 12-month longitudinal study by Falgayrac *et al.* [25] tracked diagenetic modifications in human bone specimens interred under controlled conditions, demonstrating that the mineral phase undergoes a dissolution–recrystallization mechanism characterized by decreasing carbonate content and increasing crystallinity, while collagen fibres progressively fragment, as evidenced by diminishing cross-link signatures. These findings corroborate the feasibility of Raman-based PMI estimation but underscore the necessity of environment-specific calibration datasets.

V. SPECIES DISCRIMINATION: HUMAN VERSUS NON-HUMAN BONE

A recurring challenge in forensic casework is the determination of whether recovered osseous material is of human or animal origin a question that must often be resolved before a full forensic investigation is authorized [26]. Morphological criteria suffice when large, intact skeletal elements are available, but fragmented, burned, or commingled assemblages frequently defy visual classification.

This analytical gap is addressed by Raman spectroscopy through the exploitation of species-dependent differences in bone composition and crystallography. Hernández *et al.* [27] demonstrated that the first two principal components of Raman spectra acquired from bovine, porcine, turkey, and chicken bone accounted for over 85% of spectral variance and achieved complete separation in a two-dimensional scores plot. The spectral features driving the discrimination were the relative

intensities of the phosphate and carbonate bands (reflecting interspecies variation in mineral stoichiometry) and the amide I and CH_2 deformation bands (reflecting differences in collagen cross-linking and lipid content).

Extending these findings to a forensically more relevant comparison human versus large-mammal bone remains an active area of investigation. Preliminary data from de Oliveira *et al.* [28] suggest that human cortical bone can be distinguished from porcine and bovine analogues on the basis of subtle differences in the carbonate-to-phosphate ratio and the crystallinity index, although the magnitude of these differences is smaller than those observed across more phylogenetically distant taxa. The integration of machine-learning classifiers (support vector machines, random forests) trained on Raman spectral libraries is expected to sharpen discriminative power considerably [29].

VI. THERMAL HISTORY RECONSTRUCTION OF BURNED AND CREMATED REMAINS

Fire-related fatalities whether accidental, suicidal, or homicidal confront forensic practitioners with remains whose morphology and chemistry have been profoundly altered by heat [30]. Estimating the temperature to which bone was exposed, and the duration of that exposure, provides critical context for reconstructing the circumstances of death and for assessing the viability of downstream molecular analyses.

Spectral transformations undergone by bone upon heating have been well documented and follow a broadly reproducible sequence [11,12]. Below approximately 300 °C, the Raman spectrum remains qualitatively similar to that of unheated bone, though the amide bands broaden and diminish. Between 300 and 700 °C, collagen is progressively pyrolyzed: the amide I and III bands vanish, carbonaceous residues give rise to the characteristic D and G bands of disordered carbon near 1350 and 1580 cm-1, and the phosphate ν1 band narrows as crystallinity increases. Above 700 °C, organic components are entirely eliminated, the D and G bands disappear, and the residual spectrum is that of a highly crystalline, carbonate-depleted apatite [31].

Mamede *et al.* [32], in a methodological review of heat-induced diagenesis, compiled the vibrational criteria by which the spectral trends measured under controlled laboratory conditions can be recognised in real-world burned remains. The OH-lib band, the ν(OH) stretching mode, and the $\nu_4(PO_4^{3-})$ bending region were identified as particularly informative biomarkers, each displaying quantitative relationships with maximum exposure temperature that could be calibrated for forensic use.

Spatially offset Raman spectroscopy (SORS) has introduced an additional capability: the non-invasive interrogation of subsurface bone layers without sectioning [33]. Because heat-induced diagenesis propagates inward from the periosteal surface, the outer cortex and the inner medullary region of a single bone cross-section may record different thermal histories. SORS, by collecting photons that have diffused through several millimetres of tissue, can retrieve this depth-resolved information in a fully non-destructive manner a decisive advantage when dealing with irreplaceable forensic evidence.

VII. BIOLOGICAL PROFILING FROM DENTAL HARD TISSUES

Teeth, by virtue of their exceptional resistance to post-mortem degradation, occupy a privileged position in forensic identification. Dental hard tissues survive conditions burial, immersion, fire, chemical assault under which bone and soft tissue are destroyed, and they retain molecular information over centuries to millennia [34].

Age estimation from dentin has attracted sustained attention. The progressive deposition of secondary dentin, the accumulation of mineral within dentinal tubules (sclerosis), and the post-translational modification of collagen all leave spectroscopic traces. Gamulin *et al.* [35] built a principal component regression (PCR) model from the Raman spectra of human premolars and achieved a mean absolute error of approximately 5 years across a sample spanning the second through eighth decade of life. The spectral features most strongly correlated with chronological age were the phosphate ν_1 band intensity (increasing with mineralization) and the amide I envelope (evolving with collagen cross-linking).

A more recent breakthrough concerns sex determination from dental enamel. Traditional methods for sex estimation from teeth rely on odontometric measurements whose accuracy rarely exceeds 80% [36]. Banjšak *et al.* [37] demonstrated, using Raman micro-spectroscopy of apical dentin, that spectral differences between male and female donors attributed to subtle variations in mineral crystallinity and organic content could be exploited to classify sex with an accuracy exceeding 90% in a proof-of-concept dataset. Whether this performance generalizes across populations and age cohorts remains to be established, but the result opens a genuinely novel avenue for non-destructive biological profiling.

Beyond age and sex estimation, the forensic potential of Raman analysis of dental tissues is extended to the detection of exogenous substances. Raman spectroscopy has demonstrated the capacity to identify cocaine hydrochloride deposited on human nail and tooth surfaces, even when obscured by nail varnish or enamel pellicle, raising the prospect of drug-exposure screening on post-mortem dental specimens without sacrificing material for toxicological assay [38].

VIII. PRE-SCREENING FOR DNA RECOVERY POTENTIAL

DNA extraction from skeletal remains is expensive, time-consuming, and by no means guaranteed to yield amplifiable template. In mass-disaster or mass-grave contexts, where hundreds or thousands of bone fragments may require processing, the ability to triage specimens according to their DNA recovery potential would represent a significant operational efficiency gain [17].

The rationale for Raman-based pre-screening rests on the empirical observation that collagen preservation and DNA preservation are broadly correlated: both macromolecules occupy the organic compartment of bone and are subject to similar degradative mechanisms (hydrolysis, microbial attack, oxidation) [8,18]. A Raman spectrum acquired in under one minute can quantify the mineral-to-matrix ratio and the collagen quality index, thereby providing an indirect but rapid estimate of the likelihood that extractable DNA remains.

Pestle *et al.* [39] evaluated this approach on a collection of archaeological specimens and concluded that Raman pre-screening correctly identified the majority of specimens from which no DNA could subsequently be recovered, thereby avoiding futile extraction attempts. The predictive power was highest for specimens at the extremes of preservation (excellent or very poor) and weakest for intermediate cases, where overlapping spectral characteristics made binary classification unreliable. Handheld Raman instruments have since been validated for this purpose in field settings, bringing

the screening step to the point of recovery rather than requiring laboratory access [19].

IX. DETECTION AND IDENTIFICATION OF FORENSIC BODY FLUIDS

Although body-fluid identification falls more squarely within the remit of forensic serology than forensic anthropology sensu stricto, the overlap is substantial whenever skeletal remains are associated with biological staining blood, for instance, in cases of sharp-force trauma to bone [40]. Surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful technique for this purpose.

In SERS, the enhancement mechanism is governed by the interaction of analyte molecules with nanostructured metallic substrates (typically gold or silver), which amplify the Raman signal by factors of 104 to 108 through electromagnetic and chemical enhancement [41]. For bloodstain analysis, the target chromophore is the heme moiety of hemoglobin, whose Raman fingerprint the pyrrole ring breathing mode at 754 cm^{-1} and the Cβ–Cβ stretches near 1513 and 1543 cm^{-1} provides unambiguous identification even from highly diluted or aged stains [42].

Köroğlu *et al.* [43] demonstrated that silver-nanoparticle-based SERS could detect blood at dilutions of 1:100,000 on a variety of porous and non-porous substrates, with no spectral interference from the underlying material. The technique also discriminates blood from other red-colored substances (paint, dye, food residues) that confound presumptive color tests. A recent systematic review by Dotti *et al.* [44] concluded that SERS body-fluid analysis is technically mature and could complement or, in some scenarios, replace conventional serological screening, with the added advantage that the same specimen remains available for subsequent DNA profiling.

X. PORTABLE INSTRUMENTATION AND FIELD DEPLOYMENT

From a practical standpoint, the forensic relevance of any analytical technique is substantially enhanced when it can be deployed at the scene of investigation rather than being confined to a distant laboratory. The miniaturization of diode-pumped solid-state lasers, compact diffraction gratings, and CCD detectors has enabled the development of handheld Raman spectrometers weighing less than two kilograms, with spectral resolution adequate for most forensic-anthropological applications [45].

Field validation studies have demonstrated that portable devices can reliably reproduce the spectral features critical for species discrimination, PMI estimation, and collagen quality assessment, although with somewhat lower signal-to-noise ratios than benchtop systems [7,19]. Fluorescence interference remains the principal limitation: biological specimens, soil contaminants, and organic degradation products frequently exhibit broadband fluorescence that overwhelms the Raman signal. Mitigation strategies include the use of longer-wavelength excitation (785 nm or 1064 nm), time-gated detection, and computational baseline correction algorithms [46].

A second practical constraint concerns the spatial resolution of handheld devices. Benchtop Raman microscopes achieve lateral resolution on the order of 1 μm, permitting analysis of individual osteons, cement lines, or dentinal tubules. Portable spectrometers typically interrogate an area of 50–200 μm, averaging over heterogeneous tissue microstructure and potentially obscuring diagnostically important spatial variation [45]. For applications that demand microstructural resolution Raman mapping of heat-altered bone cross-sections, for example laboratory-based confocal instruments remain indispensable.

XI. ANALYTICAL CHALLENGES AND METHODOLOGICAL CONSIDERATIONS

Despite the considerable promise outlined in the preceding sections, several analytical and methodological challenges temper the enthusiasm surrounding Raman spectroscopy in forensic anthropology. A candid appraisal of these limitations is essential if the technique is to gain acceptance within the medico-legal community, where evidentiary standards demand rigorous validation [47].

Fluorescence interference, as noted, represents the single most pervasive obstacle. Post-mortem specimens, particularly those recovered from burial, are frequently contaminated with humic acids, iron oxides, and microbial metabolites, all of which fluoresce under visible-wavelength excitation. While near-infrared excitation at 1064 nm largely eliminates fluorescence, the Raman scattering cross-section is inversely proportional to the fourth power of wavelength, necessitating longer acquisition times or higher laser powers neither of which is desirable for rapid forensic screening [46].

Standardization constitutes a second, perhaps more insidious, concern. Published studies employ a bewildering diversity of laser wavelengths (532, 633, 785, 830, 1064 nm), spectral resolutions, acquisition parameters, and data-processing pipelines, making interstudy comparison and meta-analysis difficult [24]. The forensic Raman community would benefit considerably from the adoption of consensus protocols specifying minimum reporting standards for acquisition conditions, spectral preprocessing (baseline correction, normalization), and statistical validation procedures.

Bone heterogeneity, often underestimated, poses further challenges. Raman spectra vary measurably between cortical and trabecular bone, between periosteal and endosteal surfaces, between different skeletal elements, and across age, sex, and nutritional status [4]. Forensic specimens, by definition, are of unknown provenance, and the absence of controlled baseline data can introduce systematic bias into quantitative models. The construction of large, population-representative Raman reference databases analogous to the DNA databases that underpin forensic genetics would mitigate this vulnerability but requires a coordinated, multicenter effort that has yet to materialize.

XII. CHEMOMETRIC AND MACHINE-LEARNING ANALYSIS OF FORENSIC RAMAN DATA

None of the forensic questions considered in this review can be answered by inspecting a single Raman band. Post-mortem interval, thermal exposure, species origin and collagen preservation are all encoded in subtle, mutually correlated changes distributed across a spectrum that typically comprises one to two thousand intensity values, while the number of specimens available in a forensic study is almost always smaller, often by two or three orders of magnitude, than the number of variables measured. Multivariate analysis is therefore not an optional refinement but a structural necessity, and the credibility of a Raman-based forensic inference rests as much on the statistical pipeline as on the spectroscopy itself [48].

Every such pipeline begins with preprocessing, and the choices made at this stage propagate through everything that follows. Cosmic-ray spikes must be removed; the fluorescence background must be subtracted, most commonly by asymmetric

least-squares or iterative polynomial fitting; the spectrum must be smoothed and, where appropriate, differentiated, the Savitzky–Golay filter remaining the standard tool for both operations [49]; and the intensities must be normalized, either by vector or standard-normal-variate scaling or with respect to a band assumed to be invariant [50]. Because preprocessing can manufacture apparent group separation as readily as it can reveal genuine separation, forensic reporting should specify every step and its parameters [20].

Principal component analysis is the customary first step. By projecting the spectra onto the orthogonal directions of maximum variance, PCA compresses one or two thousand variables into a handful of scores, three to five components typically accounting for 90 to 98 per cent of the total variance in bone spectral datasets, and the corresponding loadings can be read directly in spectroscopic terms: a loading dominated by the 960 and 1070 cm^{-1} features points to mineral reorganization, one dominated by the amide bands to collagen degradation. Its principal virtues are that it is unsupervised, and therefore incapable of manufacturing a group structure that is not present in the data, and that it exposes outliers and batch effects. Its principal limitation is equally consequential: variance is not discrimination. In forensic datasets the largest source of variance is frequently instrumental (focus, laser power, residual fluorescence) or anatomical rather than the factor under investigation, and a score plot that fails to separate two classes does not establish that they are inseparable.

Partial least-squares methods address precisely this weakness by extracting latent variables that maximize covariance with the response rather than variance within the predictors [51]. In the discriminant formulation (PLS-DA), used for binary or small multi-class problems such as human versus non-human bone or the identification of a body fluid, three to eight latent variables are usually sufficient, and sensitivities and specificities above 90 per cent are commonly reported. In the regression formulation (PLS-R, or the closely related principal component regression) the response is continuous, which makes it the natural choice for post-mortem interval and age estimation: Baptista *et al.* [14] obtained errors of roughly ±2 years over a 0 to 30 year burial range by this route, and Gamulin *et al.* [35] applied principal component regression to the Raman spectra of teeth for the determination of age. Two precautions are essential. The number of latent variables must be selected by cross-validation rather than by maximizing fit, and the significance of the model should be confirmed by permutation testing, since PLS-DA applied to random data with more variables than specimens will reliably produce an apparently excellent separation.

Ensemble and kernel methods have gained ground over the past decade. Random forests aggregate the predictions of a large number of decision trees, each grown on a bootstrap resample of the specimens and, at every split, on a random subset of the spectral variables [52]. Three properties make them attractive in a forensic context: they require little tuning, they provide an internal error estimate from the out-of-bag specimens, and they return a variable-importance ranking that maps back onto identifiable Raman shifts. This last property matters more than it may appear, since a model whose decisions can be traced to specific and chemically interpretable bands is considerably easier to defend under cross-examination than one that cannot. Support-vector machines, which construct a maximum-margin separating surface in a kernel-induced feature space, are well suited to the low-sample, high-dimension regime characteristic of forensic Raman work; Sieverts *et al.* [53] reported validation accuracies of up to 98.4 per cent for the discrimination of bone types after reducing the spectra to a limited set of diagnostic peaks.

Artificial neural networks, and in particular one-dimensional convolutional architectures, dispense with much of the preceding pipeline by learning the relevant spectral features, and to a considerable extent the preprocessing itself, directly from the raw data [54,55]. Classification accuracies above 95 per cent are now routinely reported for Raman-based biological classification tasks, and the approach is demonstrably more robust to instrumental drift than fixed-feature methods [48]. The cost is a requirement for training sets of the order of 10^3 to 10^4 spectra, which no forensic-anthropological study has yet assembled, together with a loss of interpretability that is not trivial in a legal setting. Data augmentation, transfer learning from adjacent domains, and hybrid architectures that constrain the network to physically meaningful spectral regions are the routes by which this constraint is most likely to be relaxed.

One caveat applies to all of the performance figures cited above and, indeed, to most of those found in the literature. The great majority are obtained by randomly partitioning a pool of spectra into training and test sets. When several spectra have been acquired from each specimen, which is the norm, a random split places replicate measurements of the same individual on both sides of the partition, and the resulting accuracy measures the reproducibility of the instrument at least as much as the generalizability of the model. Partitioning must therefore be carried out at the level of the individual, not of the spectrum. Beyond this, forensic deployment requires an external test set acquired independently of the training data, ideally on a different instrument and by a different operator; the reporting of confidence intervals and of the full confusion matrix rather than a single accuracy figure; and, ultimately, inter-laboratory validation of the kind prescribed by established forensic method-validation frameworks [47,56,57]. Table III summarizes the methods discussed here together with the figures of merit reported for them and the reservations that attach to each.

Table III.

CHEMOMETRIC AND MACHINE-LEARNING APPROACHES APPLIED TO FORENSIC RAMAN DATA, WITH REPORTED FIGURES OF MERIT AND THEIR PRINCIPAL CAVEATS.

Method (category)	Typical forensic application	Reported figures of merit	Principal caveat
PCA and hierarchical cluster analysis (unsupervised)	Exploratory grouping, outlier detection, dimensionality reduction	Three to five components typically capture 90–98 % of the spectral variance	Variance is not discrimination; frequently dominated by instrumental effects
PCA-LDA (supervised, after unsupervised reduction)	Species and tissue discrimination	Accuracies commonly reported in the 85–95 % range	Highly sensitive to the number of components retained; overfits readily
PLS-DA (supervised classification)	Human versus non-human bone; body-fluid identification	Sensitivity and specificity frequently above 90 %	Requires permutation testing; optimistic when variables outnumber specimens
PLS-R and principal component regression (supervised regression)	Post-mortem interval and age estimation	Errors of about ±2 years over 0–30 years of burial [14]; age determination from dental spectra [35]	Calibration is site-, population- and matrix-specific

Support-vector machine (supervised classification)	Bone-type and species discrimination	Validation accuracy up to 98.4 % on reduced peak sets [53]	Kernel and hyperparameter selection; limited interpretability
Random forest (ensemble classification)	Classification with an interpretable ranking of diagnostic bands	Accuracy comparable to SVM, with an out-of-bag error estimate [52]	Can overfit correlated spectral variables; importance ranking is not causal
1D convolutional neural network and related deep architectures (representation learning)	Classification from raw spectra; robustness to instrument transfer	Above 95 % commonly reported on internally partitioned datasets [54,55]	Requires 10^3–10^4 spectra; limited interpretability in a legal setting

XIII. EMERGING PERSPECTIVES AND FUTURE DIRECTIONS

It is anticipated, on the basis of several converging technological and methodological trends, that the forensic-anthropological applications of Raman spectroscopy are poised to expand and mature over the coming decade. Raman hyperspectral imaging, which generates spatially resolved maps of chemical composition across an entire tissue section, offers the possibility of visualizing diagenetic fronts, heat-alteration gradients, and pathological lesions with micrometer-level precision [58]. Combined with automated image-analysis algorithms, this modality could substantially reduce the subjectivity inherent in conventional histological assessment.

Deep-learning architectures (convolutional neural networks, autoencoders) applied to Raman spectral classification represent another frontier with immediate forensic relevance. Early studies indicate that neural networks trained on raw, unprocessed spectra can outperform classical chemometric approaches (PCA-LDA, PLS-DA) in tasks such as species discrimination and PMI estimation, while being more robust to instrumental variability and sample heterogeneity [48]. Once again, the critical bottleneck is found to be the availability of large, well-curated training datasets.

Tip-enhanced Raman spectroscopy (TERS), which combines atomic-force microscopy with plasmonic enhancement, achieves spatial resolution below 10 nm and could, in principle, probe individual collagen fibrils or mineral crystallites within a bone section [59]. While TERS remains a laboratory curiosity at present, its forensic potential for example, in characterizing nanoscale diagenetic alteration at the collagen–mineral interface warrants monitoring.

Finally, the coupling of Raman spectroscopy with complementary techniques deserves emphasis. Multimodal approaches that integrate Raman data with FTIR, X-ray diffraction, or elemental mapping (micro-XRF) have consistently yielded richer and more reliable forensic inferences than any single technique in isolation [60]. The development of integrated sensor platforms combining Raman and fluorescence channels in a single handheld device would represent a particularly valuable advance for field forensic anthropology.

XIV. CONCLUSION

Raman spectroscopy has established itself as a versatile, non-destructive, and information-rich analytical technique with demonstrated applicability across a broad spectrum of forensic-anthropological questions. From the estimation of post-mortem intervals to the discrimination of species origin, from the reconstruction of thermal histories to the biological profiling of dental remains, the published literature consistently reveals a technique whose strengths molecular specificity, minimal sample preparation, compatibility with subsequent analyses, and portability align closely with the operational requirements of modern forensic practice. This assessment, however, calls for an important qualification. What the literature establishes is an analytical capability, demonstrated for the most part on curated specimen sets under controlled laboratory conditions; it is not, or not yet, a validated routine forensic application. The distinction is not semantic. Post-mortem interval estimation, species discrimination, biological profiling from dental tissues and bone quality assessment all remain, on the published evidence, at the proof-of-concept or early-validation stage.

Four requirements separate the present state of the field from routine casework deployment. First, validation on larger and more representative datasets, sampled across the demographic and taphonomic diversity that forensic practice actually encounters. Second, the development and adoption of standardized acquisition and spectral-processing protocols, without which results obtained in different laboratories cannot be meaningfully compared. Third, independent inter-laboratory validation studies, which remain almost entirely absent from the forensic Raman literature. Fourth, evaluation under authentic casework conditions, on specimens of unknown provenance and uncontrolled taphonomic history, rather than on experimental series in which the ground truth is known in advance. These requirements are not peculiar to Raman spectroscopy; they restate, for this technique, criteria that have been formulated for forensic methods in general [1,47,56,57].

For all its promise, the technique has not yet reached the level of maturity required for routine courtroom admissibility. What remains to be addressed is the absence of standardized protocols, the shortage of population-level reference databases, and the sensitivity of spectral parameters to confounding environmental variables collectively defining the path that remains to be travelled. What the evidence does support, with reasonable confidence, is that Raman spectroscopy will increasingly serve as a complementary pre-screening and triage tool one that directs the allocation of more expensive and destructive downstream analyses toward the specimens most likely to yield informative results.

A coordinated, international effort to build consensus-driven protocols, curate large spectral reference libraries, and validate Raman-based models against independent forensic casework data would accelerate the integration of this technique into the forensic anthropologists standard toolkit. The technological trajectory smaller instruments, faster acquisitions, smarter algorithms are firmly in place; what is needed now is the methodological rigour to match it.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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