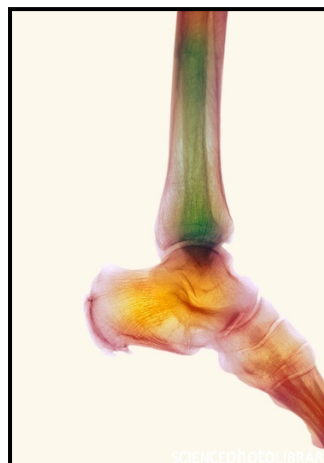


## Medical Applications of Deep Raman Spectroscopy



**Non-invasive disease diagnosis, where the patient is assessed without penetrating the body, is as compelling as it can be difficult. This note highlights the prospects for detection of diseased tissue *in vivo* through healthy tissue using new spectroscopic techniques.**

An attractive goal in biomedical research is the development of a safe non-invasive method for monitoring deep inside living tissue, e.g., in bone disease diagnosis and cancer detection. Until recently conventional Raman spectroscopy, which provides excellent chemical information, had been restricted to depths of only several hundred microns below the tissue surface. Thus many tissue components, such as veins, bones and subsurface cancerous tissue were inaccessible without invasive methods. The advent of Spatially Offset Raman Spectroscopy (SORS) has allowed the non-invasive probing of such deep tissue for the first time.<sup>i,ii</sup>

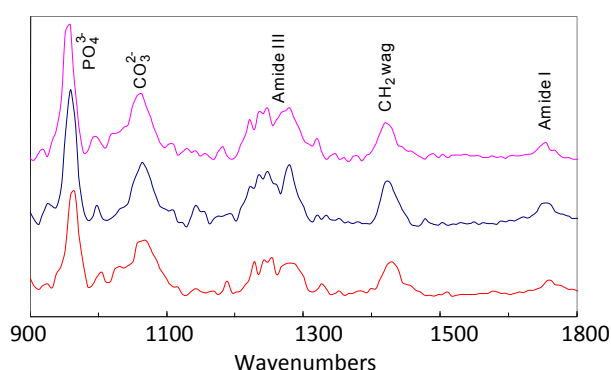


Figure 1: Non-invasive SORS spectra of human bone *in-vivo* measured at the distal phalanx of thumb.

Previous research,<sup>iii</sup> restricted to excised tissue, illustrated the considerable power of Raman spectroscopy to analysing bone matrices. The development of deep sub-surface Raman techniques provides a host of new opportunities, including the

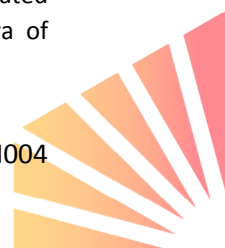
non-invasive diagnosis of brittle bone disease and osteoporosis.<sup>iii-vi</sup>

Osteoporosis is currently diagnosed by detection of mineral content (phosphate) using Dual-Energy X-ray Absorptiometry (DEXA), which provides an accuracy of only 60 to 70% in predicting osteoporotic fracture. This is believed to be due to its inability to probe the 1/3 organic (collagen) component that contributes significantly to bone strength.

The first non-invasive Raman study of bone matrix was reported by Draper *et al*,<sup>iv</sup> who successfully detected *osteogenesis imperfecta* (brittle bone disease) in mouse limbs through  $\approx 1$ mm of overlying soft tissue by comparing relative Raman intensities of the collagen and mineral components. Although this work was a major milestone, instrumental complexity and high laser intensities made it too restrictive.

The SORS method<sup>i</sup> has significant benefits to non-invasive Raman analysis. The first use of SORS for non-invasive spectroscopy of bones was reported by Schulmerich *et al*<sup>vii</sup> who obtained Raman spectra of bone from depths of several millimetres in animal and human cadavers. The subsequent use of a ring illumination geometry<sup>ix</sup> increased the quality of Raman spectra and penetration depth further and succeeded in determining a potential indicator of the presence of osteoporosis,<sup>vi</sup> through 4mm of soft tissue with better than 8% accuracy.

Parallel research by Matousek *et al*<sup>x</sup> demonstrated the basic feasibility of obtaining Raman spectra of



bones from humans *in vivo* (see Fig. 1). Improvement of the quality of *in-vivo* Raman spectra are offered by the inverse SORS approach,<sup>viii,ix,xv</sup> allowing substantial improvement in both sensitivity and penetration depth (see Fig. 2).

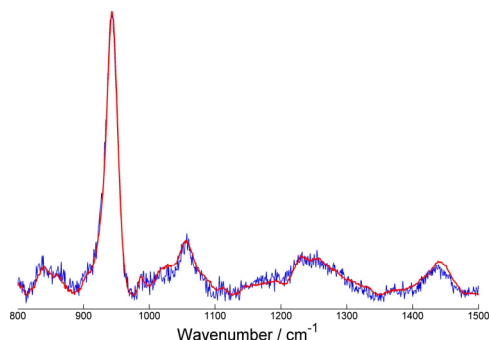


Figure 2: Raman spectrum of bone (blue) by inverse SORS through 3mm of soft porcine tissue. The Raman spectrum of bare bone is shown in red.

Another rapidly developing area of deep Raman spectroscopy is the non-invasive identification of calcifications within breast tissue. This opens up the prospect of non-invasive characterisation of malignant and benign lesions, adding diagnostic power to conventional techniques such as X-ray mammography. Presently, the detection of suspected calcifications by X-ray is typically followed by a needle biopsy, which in 70-90% of cases detects benign lesions.

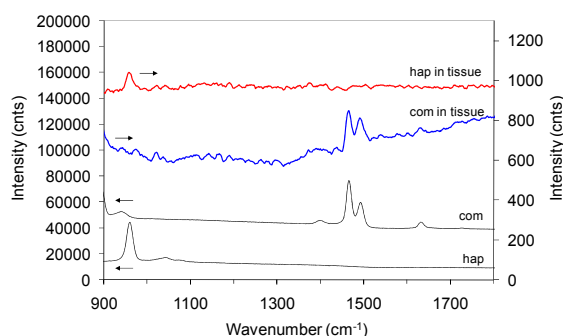


Figure 3: Raman spectra of two types of calcified material (red and blue) recovered from a 16mm-thick chicken tissue phantom conveying calcium salt type. Reference spectra of the calcifications are in black.

The potential for the non-invasive characterisation of the salt form of calcifications buried deep within

tissue was conceptually demonstrated by Baker *et al.*,<sup>xi</sup> Stone *et al.*<sup>xii</sup> and Matousek *et al.*<sup>xiii</sup> It is suggested that benign and malignant lesions can be characterised by the presence of calcium oxalate dihydrate and calcium hydroxyapatite, respectively.

Transmission Raman achieved a high penetration depth due to the intrinsic suppression of skin fluorescence. An additional benefit is that the signal strength in transmission Raman spectroscopy is independent of the depth of calcifications for a given overall tissue thickness.<sup>xiii</sup> The results of a feasibility study<sup>xiii</sup> are depicted in Figure 3 where a thin calcified material layer (100-300 $\mu$ m) was placed within the middle of chicken tissue. For both calcification types, recognisable chemical signatures were obtained. Subsequent application of chemometric methods led to the recovery of calcification signals in phantom samples from 27mm of porcine tissue.<sup>xiv</sup>

Biomedical applications of deep Raman spectroscopy have been reviewed comprehensively elsewhere.<sup>xv</sup>

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