Noninvasive Raman tomographic imaging of canine bone tissue

Matthew V. Schulmerich,a Jacqueline H. Cole,a Kathryn A. Dooley,a Michael D. Morris,a∗
Jaclynn M. Kreider,b Steven A. Goldstein,b Subhadra Srinivasan,c and Brian W. Poguec
aUniversity of Michigan, Department of Chemistry, Ann Arbor, Michigan 48109-1055
bUniversity of Michigan, Department of Orthopaedic Surgery, Orthopaedic Research Laboratories, Ann Arbor, Michigan 48109-2200
cDartmouth College, Thayer School of Engineering, Hanover, New Hampshire 03755

Abstract. Raman spectroscopic diffuse tomographic imaging has been demonstrated for the first time. It provides a noninvasive, label-free modality to image the chemical composition of human and animal tissue and other turbid media. This technique has been applied to image the composition of bone tissue within an intact section of a canine limb. Spatially distributed 785-nm laser excitation was employed to prevent thermal damage to the tissue. Diffuse emission tomography reconstruction was used, and the location that was recovered has been confirmed by micro-computed tomography (micro-CT) images.

Keywords: tomography; Raman spectroscopy; bone; diffuse; imaging; non-invasive.

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With recent advances, diffuse tomography shows promise for in vivo clinical imaging. In principle, algorithms developed for fluorescence imaging in tissue can be applied to Raman signals. Although the Raman effect is weaker than fluorescence, the scattered signal is detectable, and thus tomography is achievable. Here we demonstrate the first diffuse tomography reconstructions based on Raman scatter.

Raman mapping and imaging are well-established techniques for examining material surfaces. Subsurface mapping of simple planar objects was reported recently using fiber optic probes with spatially separated injection and collection fibers. Noninvasive measurements of bone Raman spectra were demonstrated at depths of 5 mm below the skin.

Bone is promising for Raman tomography because the spectra are rich in compositional information, which reflects bone maturity and health. Spectroscopically measured bone composition changes have been correlated with aging and susceptibility to osteoporotic fracture. The Raman spectrum of bone mineral is easily distinguished from the spectra of proteins and other organic tissue constituents, facilitating recovery of even weak signals by multivariate techniques.

Assessments of bone quantity and quality are essential to detect and monitor fracture risk and fracture healing with disease or injury. Common sites for fracture with osteoporosis are the spine, proximal femur, and distal radius. Stress fractures are most frequently seen in the weight-bearing sites of the tibia and metatarsals. Fracture risk depends on bone geometry, architecture, and material properties, as well as the nature of applied load (magnitude, rate, and direction). As a result, noninvasive imaging and nondestructive analysis methods have been developed to assess many of these bone attributes that are increasingly important to clinical practice and basic research in orthopedics. Current clinical in vivo methods include dual-energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), magnetic resonance imaging (MRI), ultrasound, and most recently, high-resolution peripheral QCT. Ex vivo analyses of bone specimens from patients or animals have also utilized these and other techniques.

In this study, we couple micro-computed tomography (micro-CT) and diffuse optical tomography with Raman spectroscopy to recover spatial and composition information from bone tissue ex vivo. We demonstrate the first reconstruction-based recovery of Raman signals through thick tissues to yield molecular information about subsurface bone tissue. Reconstructions from transcutaneous Raman measurements are challenging, because layers of skin, muscle, fat, and connective tissue lie over the bone sites of interest. These layers have different optical properties and thus vary, scattering and polarizing the injected light.

We chose a canine model because of specimen availability and a bone size similar to human bone. We selected the tibia, a site that is clinically important and has relatively few overlying soft tissues. Measurements were made on the medial surface, where the only additional optical barrier is the crural extensor retinaculum ligament. The canine hind limb was harvested from an animal euthanized in an approved (UCUCA) University of Michigan study. The section of the limb distal to the knee was excised and scanned using in vivo micro-CT (eXplore Locus RS, GE Healthcare, Ontario, Canada). The tibia was scanned at 80 kV and 450 μA with an exposure time of 100 ms using a 360-deg scan technique. The image was reconstructed at a 93-μm voxel resolution [Fig. 1(a)].

The Raman system (785-nm excitation wavelength) and preprocessing and data analysis protocols for the noninvasive recovery of bone spectra have been described previously. Briefly, hair was removed, and glycerol was applied for optical clearing. Sixty-second acquisitions were made at 10 different ring inner diameters between 6 and 16 mm. Prior to tomographic reconstruction, a bone Raman factor was recovered transcutaneously in a backscattered geometry using data from three ring diameters that maximized bone tissue contributions to the Raman signal. After transcutaneous measurements were completed, overlying tissue was removed, and the exposed bone spectrum was obtained [Fig. 1(b)]. The cross-correlation coefficient between the transcutaneous bone factor and exposed bone spectrum was 0.97, indicating that factor recovery was successful.

The same laser, spectrophotograph, and detector were used for tomography. The specimen was mounted on a 360-deg rota-
For tomographic reconstruction, calibrations were performed using a tissue phantom (9-mm diameter Teflon sphere placed 10 mm below the surface of a cylindrical phantom (diameter 59 mm, height 44.5 mm) composed of 1% Intralipid/agarose gel. A data-scaling factor, computed as the average difference between experimental and model-generated measurements from the phantom, was subtracted from the canine tibia dataset to compensate for the model-data mismatch. This approach, which is commonly used in diffuse tomography to scale the dataset to the model and to avoid calibration errors and image artifacts, simply shifts the intensities up or down to match the model but does not affect the spatial recovery within the image.

The Raman reconstructions were based on NIRFAST (http://nir.thayer.dartmouth.edu/), a reconstruction software toolbox developed at Dartmouth for photon emission imaging. The toolbox uses a finite element model to solve a set of coupled diffusion equations, which predict the way the excitation fluence and the Raman emission fluence travel through the highly scattering media. This model, known to be approximate in small tissues, is sufficient for small animal studies with length scales similar to the dog leg. A modified iterative Newton’s method minimizing the least squares norm of model-data differences was used for reconstruction. The toolbox was modified to incorporate the line focus of the sources. Since diffuse optical image reconstruction is ill-posed and ill-conditioned, we used regularization to stabilize the inversion. The starting regularization value was 100, relative to the diagonal of the Hessian matrix to be inverted, and this value was based upon the preliminary phantom experiments, as has been done in our diffuse tomography imaging studies previously. However, the reconstruction of the Raman signal by itself did not assume any knowledge of location of inner tissues.

Raman diffuse tomography was used to reconstruct the canine hind limb section (Fig. 2). The medial-lateral limb thickness in the region of interest ranged between 24 and 45 mm. The signal/noise ratio, measured using the 

$$\text{SNR} = \frac{I_{\text{peak}}}{I_{\text{noise}}}$$

where 

$$I_{\text{peak}}$$

is the intensity of the peak Raman signal and

$$I_{\text{noise}}$$

is the noise intensity, ranged from 0 to 5.7. Two reconstructed Raman iso-surfaces were mapped onto the true location of the tibia [Figs. 2(a) and 2(b)]. Reconstructions appeared artificially spherical, possibly due to the diffuse propagation of light through tissue, which inherently results in diffuse tomography images with blurred features, and to Gaussian-shaped object recovery. The peaked intensity in the center of the bone is an artifact of this process. This problem was most apparent at high and low intensities. Isosurface thresholds between 10% [Fig. 2(a)] and 50% [Fig. 2(b)] preserved nonspherical shapes. Despite tissue thickness, the periosteal perimeter of the reconstructed image corresponded well to the true bone contour [Figs. 2(c) and 2(d)]. The highest Raman bone signal was found in the center of the bone [Fig. 2(d)], which is likely an artifact of the Gaussian-type blurring that occurs in diffuse tomography. Because light passed through both the tibia and the fibula in few projections, the fibula was not accurately reconstructed.

Without spatial priors in the reconstruction and with the limited mesh resolution, localization of the bone was imperfect. However, our Raman tomography approach provides the means to incorporate a more sophisticated reconstruction, such as with spatial priors. Although we used a diffusion model of light transport, an approximation commonly made
for tissue studies, the light was likely not truly diffuse. Future work will focus on coupling more linear transport-forward models with CT (and by extension, MRI) is feasible. Future probe designs will focus on examining composition changes in the tissue. Improved probe geometry will allow us to reduce power density and impose spatial constraints on the recovery of composition properties for tissue imaged by the clinical modality. This dual imaging technique also provides a better estimate of the Raman signal, and the spatial mapping improves the template for accurate image recovery.1,3

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