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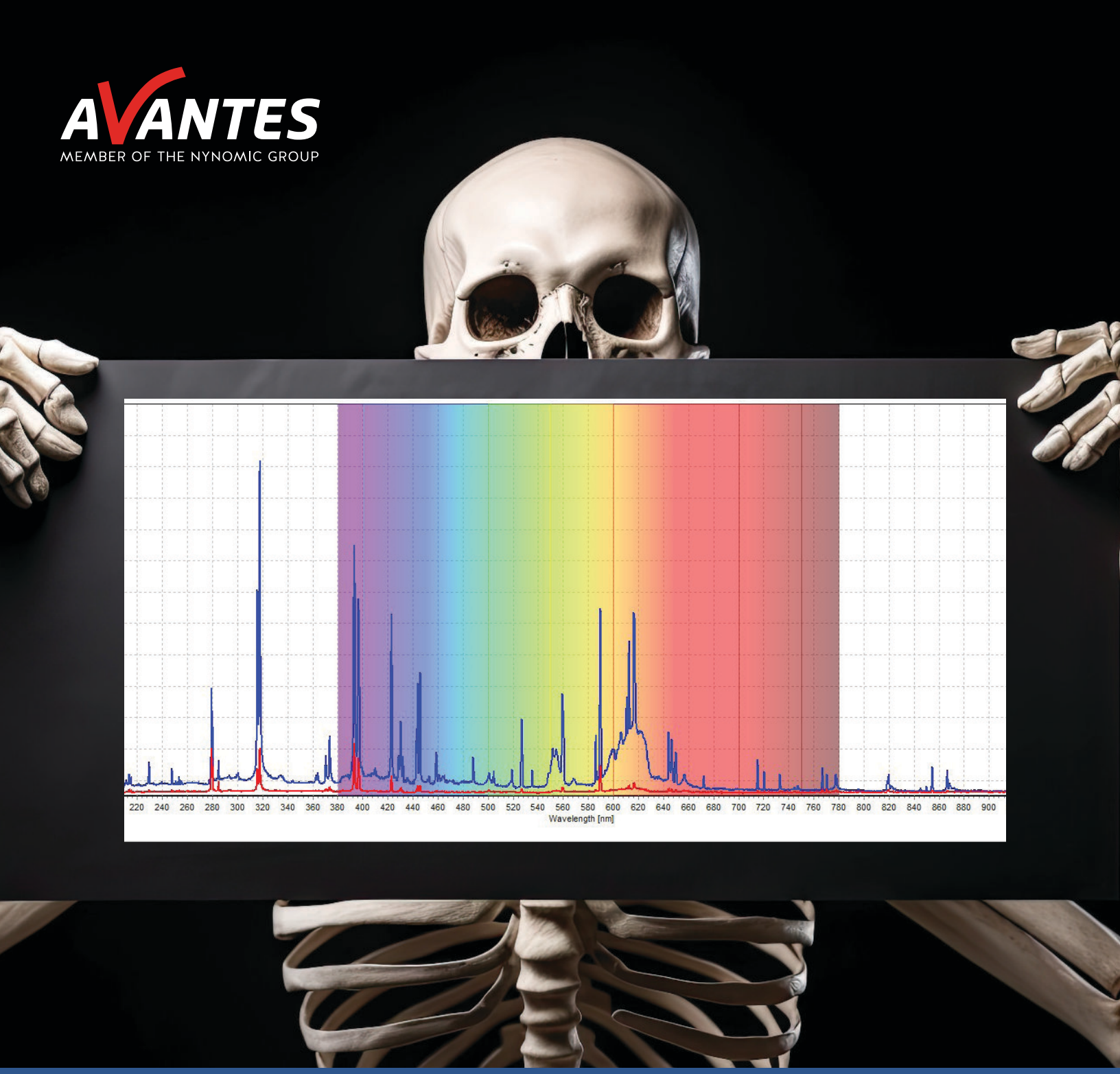

IN-HOUSE EXPERIMENT

SKELETAL SPECTROSCOPY: INVESTIGATING CORTICAL AND CANCELLOUS VARIATIONS

CONDUCTED BY KURT AMEKU

BACKGROUND INFORMATION

Along with trick-or-treating, carving pumpkins, and watching scary movies, skeletons are synonymous with the Halloween spirit. The inclusion of both skeletons and ghosts in Halloween festivities can be traced back to the Celtic festival of Samhain, from which our modern-day celebration of Halloween is derived. The holiday was believed to be a day where the boundary between the realm of the living and the realm of the dead became blurred, and the dead would return and walk amongst the living. With this "day of the dead" association, skeletons and bones in general became forever tied to Halloween. Generally, bones are divided into two categories: cortical and cancellous bone. Cortical bone, which makes up around 80% of skeletal muscle mass, is the rigid, outer layer that is responsible for a majority of weight and structural support along with storing fat in its bone marrow cavity. Cancellous bone is the spongy, inner layer of bone that is structured in a three-dimensional lattice known as trabeculae. This matrix structure arranges along stress lines to share some load bearing responsibilities, making it highly adaptable to changes in load and stress. Additionally, the space between the lattice in cancellous bone is filled with marrow and blood vessels. While these two types of bone vary significantly in structure, mechanical properties, and function, they are almost identical in terms of composition. Both types of bone are primarily composed of calcium phosphate, also known as hydroxyapatite, collagen, and water, along with some minor and trace elements. The main difference in composition between the two types of bone is calcium content, which is lower in cancellous bone. To determine calcium content, the amount of calcium must be compared to either the total mass of the sample or to the content of another present element that remains consistent. Often, a calcium-phosphorus ratio is used with bones since both elements are present in hydroxyapatite. One such technique to measure the presence of these and other elements in a sample is laser-induced breakdown spectroscopy, or LIBS.

In this LIBS-based experiment, our objective is to discern the distinct compositional disparities between cortical and cancellous bone. This technique requires the precise calibration of a laser, which, when focused on a bone sample, generates small plasma plumes. These plumes emit specific wavelengths corresponding to elements in the sample, revealing its composition. The bone sample used in this experiment (Figure 1) was cut multiple times to access both cortical and cancellous bone samples, both of which were measured using this spectroscopic technique, and individually scrutinized the resulting spectra. By matching measured peaks with the elements constituting bone composition, we directly compared the spectra to identify variations that could indicate whether a sample was cortical or cancellous bone, potentially through factors like the calcium-to-phosphorus ratio.

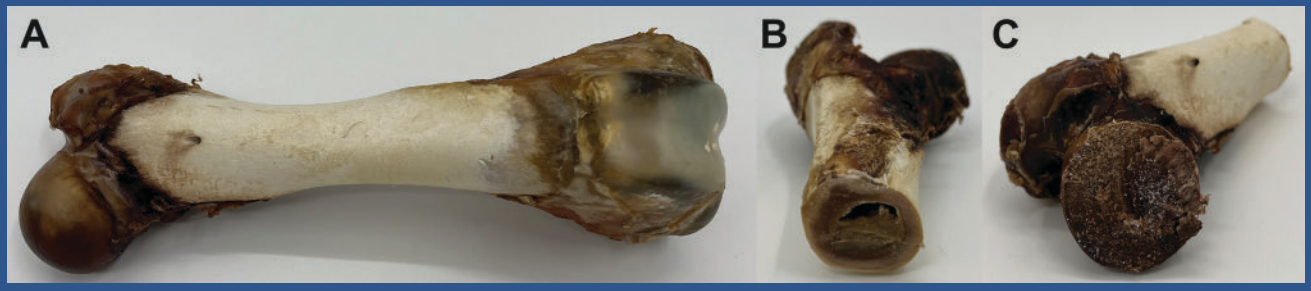

FIGURE 1: (A) Bone sample used for this experiment, post-cleaning process. The bone sample was sawn in two places, the first (B) along a radial cross-section to provide access to the cortical bone sample, and the second (C) at the ball joint to provide access to the cancellous bone sample.

DESCRIPTION OF SPECTROSCOPY SETUP

The setup for this experiment (Figure 2) utilized 2 of our new AvaSpec-PCT2048CL compact spectrometers. This compact instrument is the next-generation photonics backbone spectrometer, designed to empower a wide range of applications in various industries. This device is built using our new semi-automated manufacturing technique that ensures higher levels of consistency and reproducibility unit-to-unit. The compact spectrometer offers USB2.0 communication as well as RS232 and SPI communication protocols, a CMOS linear array detector, ultra-low stray light as low as 0.1%, and a signal/noise ratio of 375:1. Furthermore, this spectrometer can be customized with a wide range of gratings (13 total available) and the replaceable slit option is now standard for non-OEM units, which provides even more flexibility for a variety of application needs. Of the two units used in this experiment, one was optimized for the UV range at a 190-430 nm wavelength range, and the other covered a broader range in the visible and NIR range from 360-960 nm.

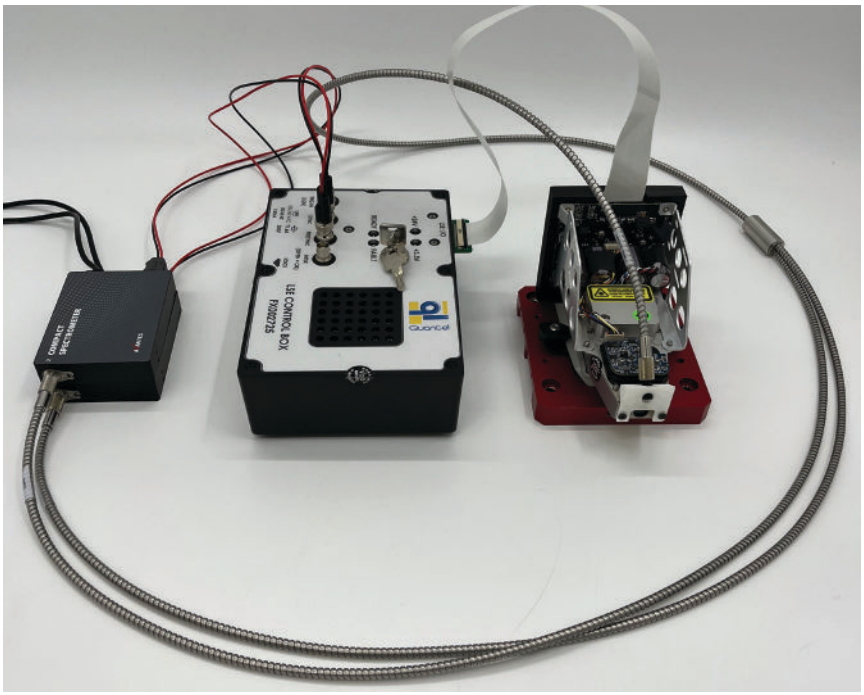

FIGURE 2 Experimental setup for bone sample measurements. The two legs of the bifurcated fiber optic cable were connected to each spectrometer while the common end was mounted and pointed at the plasma plume. Both spectrometers are individually triggered by the control box via an interface cable.

The light source used for this experiment was derived from the plasma produced by a DPSS (double pulse, single scan) Nd:YAG laser, generously provided by Lumibird. Lumibird offers a diverse selection of lasers, and this particular model is specifically designed for robust applications in challenging and unpredictable environments where temperature and vibrations would typically render most commercial lasers inoperative. This laser operates at eye-safe wavelengths, specifically at 1574 nm, with an energy of 4.5 mJ per pulse. Designed for battery operation in hand-held and mobile applications, this compact yet robust laser features maintenance free operation for years of hard use.

Other accessories used for this experiment included a 400-micron core bifurcated fiber optic cable (FCB-UVIR400-2-BX) to directly measure the plasma plume, a Quantel control box (FX002725) to power the laser and sync the laser pulses and spectrometer measurements, two custom interface cables to connect each AvaSpec-PCT20048CL to the control box, and a custom 3D-printed mount to hold the fiber optic cable at the precise angle to line up with the focal point of the laser.

DESCRIPTION OF METHODOLOGY

The bone sample used employed in this experiment was a flavored ham bone, originally intended as a canine treat, and it was purchased from a nearby pet store. In order to prep the sample for analysis, it was soaked in a soapy bath over multiple days to remove the added flavoring to the bone's outer surface, as well as break down fragments of ligaments, fat, and other tissue. After this bath, as much of the remaining tissue was cut off the bone using a razor blade, and the bone was allowed to dry overnight. The next day, the bone sample was sawn along a medial radial cross-section. This allowed measurement of the cortical bone without the defects seen on the outer layer. The bone sample was also sawn at the ball joint of the bone to allow measurement of the cancellous bone. The bone sample was placed in the firing range of the laser twice, once to measure the cortical bone and another time to measure the cancellous bone. The laser was activated using serial commands. The spectrometers were synced to the laser pulses on the hardware side using the Quantel control box and on the software side through defined settings in AvaSoft, our custom software package.

For data analysis, we used the Scope-Minus-Dark mode in AvaSoft. This is a common mode for LIBS measurements, as it subtracts the dark spectrum (what the spectrometer measures with no light source) from the raw counts (i.e., scope mode) for each wavelength. This helps minimize noise in the spectrum and better isolate and identify the plasma plume peaks. For both devices, we used an integration time of 10 milliseconds, which in most cases can be adjusted to increase or decrease the amount of light being measured at one time and affects the overall magnitude of the reported spectrum. For LIBS applications, since the plasma plume is generated and decays at such short time intervals, increasing the integration time will increase the portion of time where no light is measured and will therefore decrease the measurement amplitude. We set averaging to 1 for both units, meaning each measurement corresponds to one plasma plume instance. For a more uniform presentation of the spectra, an additional setting was used to merge the two spectrometer measurements.

TEST DATA AND RESULTS

Displayed below are the LIBS spectra of the sample in scope-minus-dark mode for each measurement.

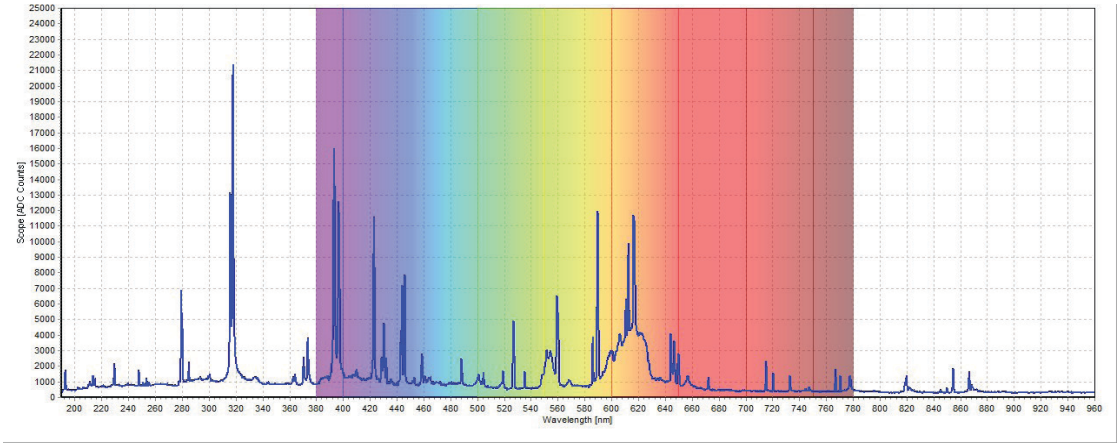

FIGURE 3: LIBS spectra of cortical bone sample.

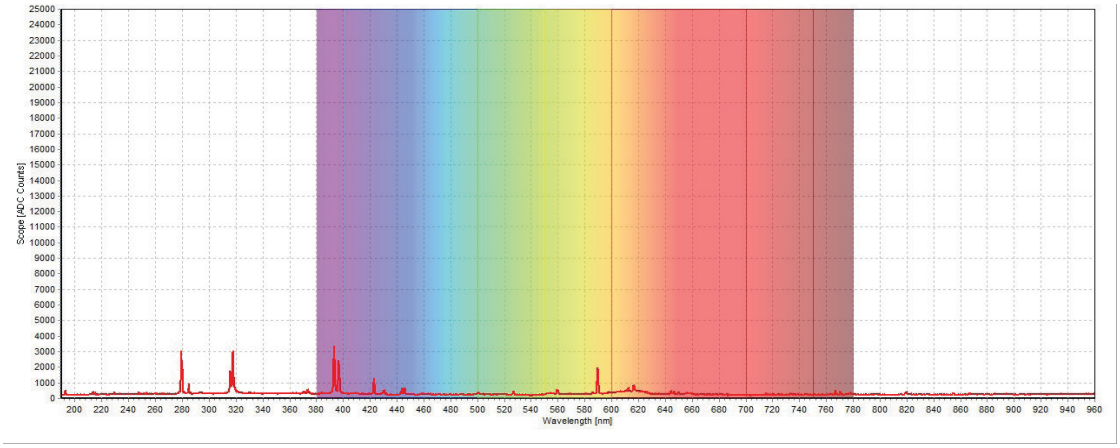

FIGURE 4: LIBS spectra of cancellous bone sample.

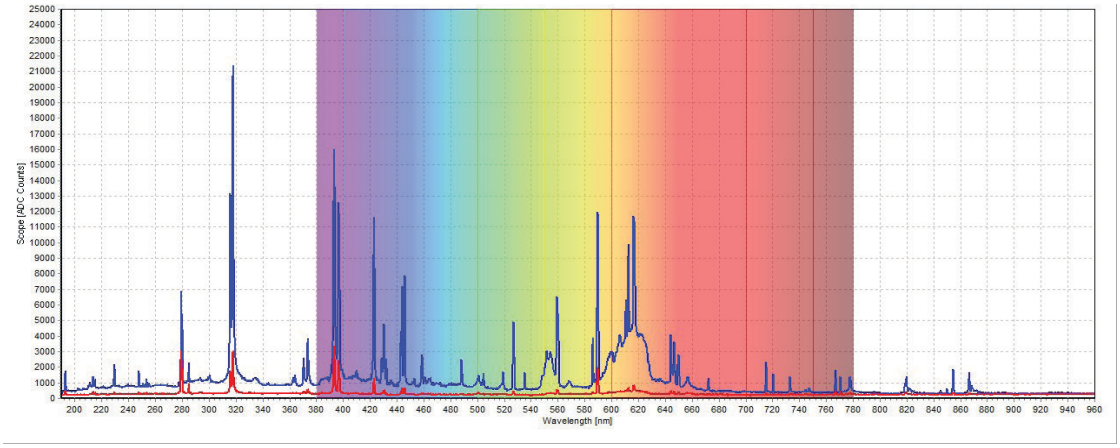

FIGURE 5: LIBS spectra of both cortical and cancellous bone samples, shown together for comparison.

ANALYSIS AND CONCLUSION

We observed that the cortical bone sample showed the largest peaks at 315.78 nm, 317.95 nm, 393.18 nm, and 396.75 nm (Figure 3). These peaks are indicative of calcium, within the measurement precision and accuracy. Other present peaks that are correlated to specific elements included additional calcium peaks (370.52 nm, 373.64 nm, 854.62 nm, and 866.61 nm), phosphorus peaks (213.51 nm, 214.95 nm, 253.40 nm, and 255.37 nm), oxygen peaks (645.43 nm and 777.80 nm), hydrogen peaks (410.15 nm and 656.83 nm), nitrogen peaks (818.69 nm, 819.97 nm, 821.88 nm, and 868.50 nm), carbon peaks (192.99 nm and 247.75 nm), magnesium peaks (279.42 nm and 285.10 nm), and potassium peaks (766.76 nm and 770.33 nm). The presence of calcium, phosphorus, oxygen, and hydrogen is consistent with the primary composition of bones being hydroxyapatite. Along with hydrogen and oxygen, the presence of carbon and nitrogen is characteristic of the portion of bone that is made up of collagen. While bone does also contain water, this was likely not measured since the sample was allowed to dry overnight. Lastly, the presence of magnesium and potassium can be attributed to their function in bone growth and development along with other minor and trace elements. In comparison, the cancellous bone sample (Figure 4) showed all the same significant peaks as the cortical bone, though all at lower magnitudes. This could be due to the fact that the structural composition of cancellous bone is much more porous than cortical bone and achieving consistently strong measurements of the sample was less feasible.

Comparing the cortical and cancellous bone samples, little difference was seen between the two in terms of peaks present (Figure 5). The magnitude of the peaks, however, gives some indication of sample differences. While the maximum calcium peak was significantly greater than the maximum phosphorus peak in the cortical bone sample (21398 counts versus 6865 counts, respectively), the same two peaks were much closer in magnitude in the cancellous bone sample (3052 counts for the calcium peak and 3050 counts for the phosphorus peak). This much smaller ratio of calcium to phosphorus demonstrates the lower calcium content present in cancellous bone compared to cortical bone. While these differences are clear in the two samples measured, further testing and analysis would need to be performed to create an algorithm that could determine if a bone sample was cortical or cancellous bone with confidence.

CONCLUSION

In summary, this experiment underscores the utility of the LIBS measurement technique for analyzing the elemental compositions of bone samples. We effectively distinguished between cortical and cancellous bone samples by comparing the calcium and phosphorus peaks, revealing their differences. Notably, the remaining peaks in each sample exhibited substantial similarities, underscoring the close elemental composition of these bone types. The AvaSpec-PCT2048CL proves to be an excellent choice for OEM applications and scenarios where a compact form factor is crucial. The Lumibird laser perfectly complements the AvaSpec-PCT2048CL for compact LIBS measurements. Both the custom interface cables and the 3D-printed cable mount highlight the capabilities of our engineering team to provide custom assemblies and solutions for customer needs. Please contact Avantes for more information on the configuration that is best suited for your data collection.

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