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Plasma Temperature-Based Tissue **Differentiation Using Laser-Induced** Breakdown Spectroscopy (LIBS) for Laser-**Guided Osteotomy**

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Abstract: Laser osteotomy enables precise and minimally invasive bone cutting but lacks real-time feedback on tissue type, increasing the risk of unintended damage to surrounding soft tissues such as tendons, muscles, nerves, and blood vessels. Accidental damage to these structures can compromise surgical outcomes and prolong recovery. To address this, we investigate the use of Laser-Induced Breakdown Spectroscopy (LIBS) for intraoperative tissue differentiation. While conventional LIBS methods rely on elemental intensity ratios, they often struggle to distinguish between tissues with similar compositions. In this study, we propose laser-produced plasma temperature as an additional stable parameter for differentiating hard and soft tissues. Experiments conducted on pig knuckle samples, selected for their anatomical similarity to human tissue, revealed clear plasma temperature-based separation between soft and hard tissues. These findings highlight plasma temperature as a valuable diagnostic parameter in LIBS—enhancing feedbackcontrolled laser surgery for better precision and tissue preservation, while also serving as a reliable input for deep learning models to enhance tissue classification accuracy. We foresee that the tissue differentiation process could be supported by temperature estimation via Boltzman plot of elements similarly in between some soft tissues.

Keywords: Laser induced breakdown spectroscopy, Plasma temperature, Laser produced plasma, Boltzmann plot.

1 Introduction

Laser osteotomy provides numerous benefits, including high precision and minimal invasiveness, yet it does not inherently relay information about the tissue being ablated [1]. Laser osteotomy in orthopaedic procedures, such as knee surgery, demands high precision to selectively remove hard tissues like bone while preserving adjacent soft tissues such as nerves, muscles, tendons, and blood vessels. Damage to these structures can result in serious complications, including impaired function, delayed healing, or long-term deficits. Preserving soft tissues is critical for maintaining joint stability and mobility in orthopaedic procedures like knee surgery. Although laser systems offer excellent precision during laser osteotomy they lack real-time tissue identification, increasing the risk of unintended damage. Integrating a mechanism that enables surgeons to recognize tissue types during ablation would significantly enhance surgical precision and safety across a range of clinical applications.

Various methods such as Photoacoustic Imaging, Diffuse Reflectance Spectroscopy (DRS), Magnetic Resonance Imaging (MRI), Optical Coherence Tomography (OCT), and Laser-Induced Breakdown Spectroscopy (LIBS) have been explored for tissue differentiation [2], [3], [4]. Among these, LIBS, which is an atomic emission spectroscopic technique that generates plasma from a sample using a focused highenergy laser pulse, enables qualitative and quantitative elemental analysis of ablated tissues [5]. While conventional

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LIBS-based tissue analysis relies on elemental intensity ratios, it often struggles to differentiate tissues with similar compositions due to effects like self-absorption and matrix variability [6]. Since the plasma is generated from the target tissue, differences in structure and composition result in measurable variations in plasma temperature [6]. This makes laser produced plasma (LPP) temperature valuable and more stable parameter for tissue differentiation, offering higher sensitivity and insight than conventional intensity ratios [7].

In this study, we explore the potential of LPP temperature as a supplementary diagnostic parameter in LIBS-based classification of hard and soft pig knuckle tissues (bone and muscle), selected for their similarity to human tissue. This thermodynamic feature enhances tissue differentiation, enabling more reliable distinction between hard and soft tissues, and supporting the development of an effective feedback-guided laser surgery system. We also anticipate that Boltzmann plot-based temperature estimation of elemental lines could aid in distinguishing closely related soft tissues.

2 Methodology

Two tissue types—bone (hard tissue) and muscle (soft tissue)—were dissected from the bisected knuckles of ex vivo domestic pigs. The samples were obtained from a local slaughterhouse; therefore, ethical approval was not required for this study. Tissues were manually excised using a scalpel into sections measuring approximately 2 × 2 cm, with thicknesses kept below 1 cm. Prior to analysis, samples were gently rinsed with saline to remove surface contaminants such as clotted blood, ensuring minimal disruption of the native tissue architecture. All LIBS measurements were performed within five hours post-slaughter, under ambient conditions with exposure to natural stray light to mimic a realistic surgical environment.

The experimental setup for nanosecond LIBS (ns-LIBS) is depicted in Figure 1. A Q-switched Nd:YAG laser (InnoLas P1853 UTL) operating at 1064 nm was employed to generate plasma, delivering pulses of 50 mJ energy and 8 ns duration at a repetition rate of 10 Hz. The laser beam was focused perpendicularly onto the tissue surface using a plano-convex lens (LA5012, Thorlabs) with a focal length of 16 cm, producing a spot size of approximately 36 μ m and yielding an irradiance of ~3.05 × 10¹¹ W/cm². Samples were placed on an XY motorized translation stage (Standa 8MT167-25BS1) and positioned precisely via a motion controller.

The plasma emission was collected using a lens-based optical collection system and delivered to a spectrometer via a $400 \mu m$ core multimode optical fiber (NA = 0.22). The

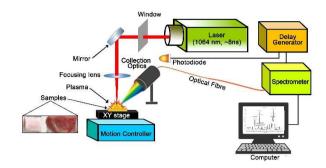


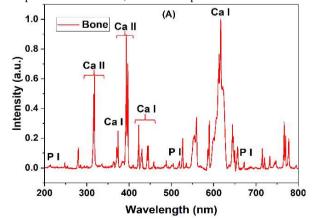
Figure 1: Schematic of the LIBS experimental setup.

spectrometer, equipped with a CCD detector, recorded spectra across the 200–900 nm range. A delay generator (Model T560, Highland Technology) synchronized the spectrometer acquisition with the laser pulse, triggered through a photodiode detecting the generated plasma light.

To ensure clean ablation and eliminate surface residues, five laser pre-pulses were applied to each measurement location prior to spectral acquisition. For each tissue type, five distinct spots were selected, with 25 laser shots per spot. The spectra obtained from each spot were averaged to produce a representative spectrum, ensuring consistency and repeatability across measurements.

3 Results and Discussion

The obtained LIBS spectra of bone and muscle tissues of pig knuckle showed specific atomic emissions resulting from the elements composing these samples. Figure 2 presents the LIBS spectra obtained from pig knuckle bone and muscle, revealing distinct atomic emission peaks corresponding to the elemental composition of each tissue type. The bone spectrum is dominated by strong emission lines of Calcium (Ca) and Phosphorus (P), showing the characteristic of its mineral-rich composition. In contrast, the muscle spectrum exhibits



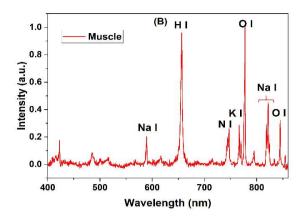


Figure 2: Mean LIBS spectrum of 25 spectra of (A) bone; (B) muscle of pig knuckle ranging from 200 – 900 nm region.

prominent peaks from Hydrogen (H), Oxygen (O), Sodium (Na), Potassium (K) and Nitrogen (N) [8]. Elemental identification was performed using the NIST atomic emission database [9].

3.1 Calculation of plasma temperature

The Boltzmann plot method was employed to estimate the electron temperature of the laser-induced plasma, assuming local thermodynamic equilibrium (LTE) conditions [10].

Under LTE, the relative population of an excited energy level *j* with respect to the ground state is given by the Boltzmann distribution:

$$\frac{N_j}{N_0} = \frac{g_j}{Z} \exp\left(-E_j/k T_e\right),\tag{1}$$

where N_j and N_0 are the population densities of level j and the ground state, respectively, g_j is the statistical weight, E_j is the excitation energy, Z is the partition function, k is the Boltzmann constant, and T_e is the electron temperature.

The intensity of the spectral line resulting from the transition from energy level j to energy level i, with transition probability A_{ji} , is expressed as;

$$I = A_{ii} N_i h \nu, \tag{2}$$

here $h\nu$ is the photon energy. Substituting the Boltzmann distribution into the intensity expression and converting frequency to wavelength yields the linearized form:

$$\ln(I\lambda/A_{ii}g_i) = -E_i/kT_e + \ln(hcN_0/4\pi Z).$$
 (3)

Using Equation (3), the Boltzmann plot of Ca II (Figure 3) is drawn using the spectral peaks at wavelengths λ_1 = 317.933 nm and λ_2 = 370.603 nm for laser-produced bone plasma and the mean electron temperature of five different spots on the sample was calculated to be 15777.69 ± 471.48K (1.360 ± 0.041eV).

Similarly, the average electron temperature of laser-produced muscle plasma was calculated to be 7542.76 \pm 781.62 K (0.650 \pm 0.067 eV) using five different spots on the sample. The considered peaks were from the slope of the Boltzmann plot of O I (Figure 3) at wavelengths λ_1 = 777.194 nm and λ_2 = 844.635 nm.

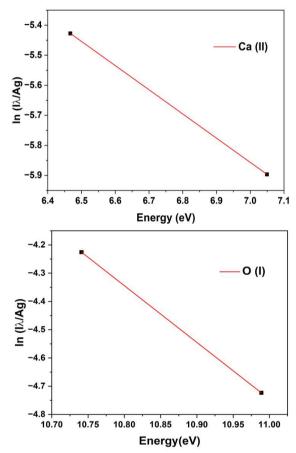


Figure 3: Boltzmann plot of Ca II and O I emission lines from a single spot in bone and muscle samples of pig knuckle, respectively.

Spot-to-spot variations in plasma temperature for bone and muscle tissues are illustrated in histogram plot (Figure 4). As shown, the temperature fluctuations across different locations on both bone and muscle samples from pig knuckle are minimal, indicating a relatively stable plasma environment. This stability is essential to ensure the accuracy and reproducibility of Laser-Induced Breakdown Spectroscopy (LIBS) measurements during laser osteotomy.

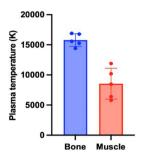


Figure 4: Plasma temperature variation of the bone and muscle samples during LIBS.

Thus, this study demonstrates the potential of LIBS as a rapid, accurate, and minimally invasive method for qualitative tissue differentiation. By evaluating plasma temperature variations between soft and hard tissues, an efficient and straightforward approach for feedback in laser surgery is presented. The proposed method shows promise for improving the clinical performance of laser systems by increasing surgical precision and minimizing the risk of soft tissue damage during complex surgical procedures.

4 Conclusion

This preliminary study confirms the feasibility of using LIBS for plasma temperature-based differentiation of biological tissues under ex-vivo conditions. Clear differences in plasma temperature were observed between soft and hard tissues of pig knuckle, highlighting the technique's potential as a feedback tool for laser-guided osteotomy. The elemental composition identified in the LIBS spectra - Ca and P in bone, and C, H, O, Na, K, and N in muscle - was consistent across all samples and aligned well with existing literature. Plasma temperature values calculated using the Boltzmann plot method for bone and muscle revealed a promising range for effective tissue differentiation. These findings suggest that the proposed approach can enhance tissue recognition accuracy during laser osteotomy and help prevent unintended damage to critical structures. Further research is needed to improve the reproducibility of plasma temperature estimation and validate its applicability across a wider range of tissue types. As part of future work, we propose integrating plasma temperature and elemental intensity ratios into deep learning models to enhance the accuracy and robustness of tissue classification using LIBS. We foresee that temperature estimation using Boltzmann plots of elemental lines could similarly support tissue differentiation among soft tissues with comparable properties.

Author Statement

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Conflict of interest: Authors declare no competing interests. Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: This statement is not applicable, as the pig bone used in this study was obtained from a local butcher shop.

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