IMPLEMENTATION OF LASER-INDUCED BREAKDOWN SPECTROSCOPY ELEMENTAL IMAGING INTO THE HISTOPATHOLOGICAL ANALYSIS OF SOFT TISSUES

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1. INTRODUCTION

Many reviews already covered the benefits of LIBS utilization in the topic of biological tissue analysis. Overall, the cost efficiency, simplicity, real-time performance and the possibility for large-scale elemental imaging provided by LIBS is vary valuable for histopathological research. In order to be able to detect variations in trace element concentration it is critical to achieve the highest possible analytical sensitivity, achieved through optimization. Even though it is often cited that LIBS does not require sample treatment, but in the case of soft tissues sample preparation can significantly influence the performance of a LIBS system [Jantzi 2016]. We are interested in imaging of the elemental layout in a section of soft tissue, thus we need to treat the sample without affecting the sample matrix. As the formalin fixation and paraffin embedding (FFPE) is routinely used by pathologists, the implementation of paraffin section imaging in LIBS opens a wide range of applications. In this work, we compared two embedding techniques, 10 μm slices of tissue embedded in paraffin on glass slides and tissues in paraffin blocks.

2. EXPERIMENTAL

All measurements were performed using the LIBS Discovery instrument. It was developed at the Central European Institute of Technology, Brno University of Technology (Brno, Czech Republic). The experimental apparatus for LIBS analysis consisted of a Q-switched Nd:YAG laser Quantel CFR Ultra (France; 532 nm, 10 ns, 20 Hz). The laser beam was focused on the sample surface by the triplet lens (Sill Optics, Wendelstein, Germany) with a focal length of 24.5 mm. Plasma emission was collected by wide-angle optics and transferred through an optical fiber to the entrance slit of a

Czerny-Turner spectrometer (SR-500i-B2-R, Andor, Northern Ireland) equipped with a grating of 1200 lines per mm and a 50 µm entrance slit. Plasma emission was obtained using a gated sCMOS detector (iSTAR-sCMOS-18F-E3, Andor, Northern Ireland). The gate width and the gain was set at 50 µs and 4000, respectively. The assignation of spectral lines was cirred out using the National Institute of Standards and Technology (NIST) database [Kramida 2018].

3. RESULTS AND DISCUSSION

3.1. Embedding

The difference between two types of tissue fixation can be seen on **Figure 1**. Here we compare elemental maps of K I 766 nm. No interference is visible in map from tissue in paraffin block as paraffin consists only of C and H. Potassium signal from the tissue clearly differentiates the kidney from the background paraffin. This also applies to Ca, Na, Mg, elements naturally present in the tissue, which from our experience can also be clearly imaged.

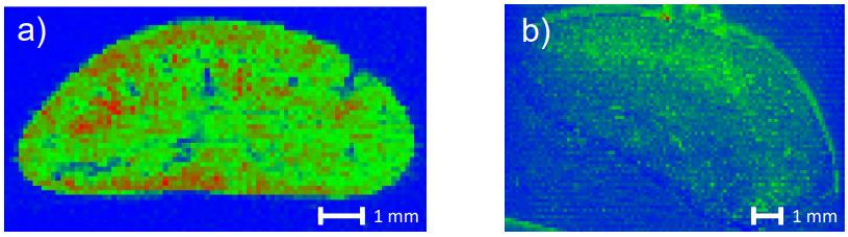

Figure 1. A typical potassium map (K I 766 nm) acquired from a) kidney in a paraffin block; b) kidney slice on a glass slide.

The elemental image of kidney slice on glass slide shows strong interference from elements present in the glass, as glass ablation cannot be totally avoided. Elements, such as Na, Ca, K cannot be properly detected. As previously mentioned, the thickness of kidney slices was 10 µm. Minimalizing the interference from the glass by cutting a thicker slice of tissue wasn't possible as the slices thicker than 10 µm were splitting. Considering preparation, both of these are easy to prepare, although kidney slices on glass slide require a few more steps before they can be used in LIBS analysis. Another benefit of paraffin embedding is that it is possible to cut fresh surface on a microtome after each measurement.

3.2. Optimization approaches

To be able to detect variations in trace elements concentration reaching LIBS limits of detection it is critical to achieve the highest possible analytical sensitivity, which should be provided by a step of optimization. Therefore, we outlined technique of optimization of elemental imaging by detecting element (potassium) present in the matrix in an amount sufficient for detection. The potassium content was 3013 (213) $mg \cdot kg^{-1}$, determined by the ICP-OES method.

We assessed the stability of the elemental signal (**Figure 2.**). Stability was determined as the relative standard deviation (RSD) of repeated measurements [Huang 2013]. The potassium signal in paraffin block is established to be 17%. These measurements were also measured on two separate occasions (red and blue datasets in **Figure 2.**) with the same experimental parameters. From the results, we assume the analysis stable in time and thus repeatable. The elemental image of potassium signal for pulse energy of 10 mJ can be seen in **Figure 3**.

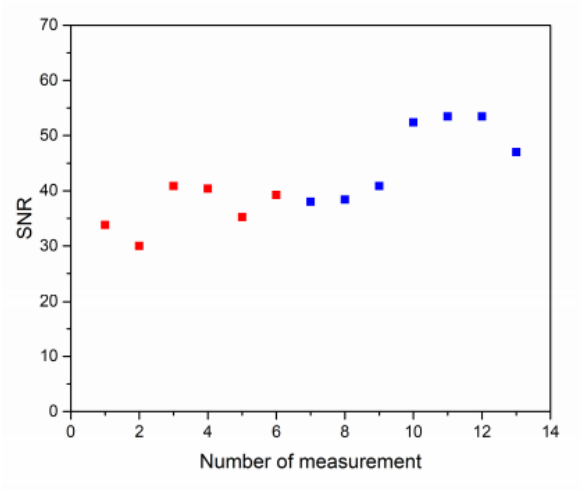

Figure 2. Stability of signal from a) mouse kidney in paraffin block (red – 21.1.2020; blue – 6.2.2020); b) It is not possible to add error lines because SNR is influenced by heterogeneous distribution of analyte concentration, therefore only the average values can be compared.

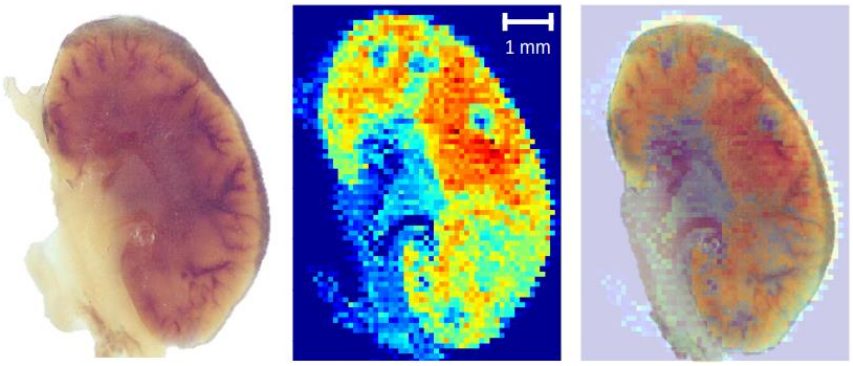

Figure 3. LIBS imaging of potassium (K I 766 nm) as acquired from a kidney sample in a paraffin block for a pulse energy of 10 mJ.

4. CONCLUSIONS

LIBS imaging of elemental distribution can provide valuable insights for the histopathological field of research. In this work, we presented comparison of two embedding techniques and two methodological approaches for the optimization of elemental imaging of soft tissues.

Embedding in paraffin blocks enables to maintain the sample structure and at the same time provides sufficient results in a view of the signal and absence of interference. Considering the stability of signal, our measurements and results from paraffin blocks are stable in time.

5. ACKNOWLEDGEMENTS

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