RECENT ADVANCES IN THE IMAGING OF BIOLOGICAL TISSUES AT THE BRNO UNIVERSITY OF TECHNOLOGY

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1. INTRODUCTION

Laser-Induced Breakdown Spectroscopy (LIBS) is an optical analytical technique with a multi-element bioimaging capability in various biologic matrices. During the years of LIBS development, the major application field has been in industry. However, during the last two decades, LIBS became a useful imaging tool in different biologic matrices, e.g. bones, tooth, skin, mammals' organs, and in the plant science. In this work, we present an overview of the Brno University of Technology (BUT) Laser Spectroscopy laboratory achievements in biotic sample analysis. The plant bioimaging started in 2007 by pioneering paper [Kaiser 2007] which established the Pb content in sunflower leaves (*Helianthus annuus*) by using the femtosecond laser (Ti:sapphire; 795 nm) with an energy of 0.1 mJ per pulse. The bioimaging of macronutrients, micronutrients, non-essential elements, and even several types of nanoparticles in various plant species and plant tissues were established in several following papers [Kaiser 2007, Galiová 2007, Galiová 2008, Kaiser 2009, Kryštofová 2009, Galiová 2011, Krajcarová 2013, Krajcarová 2017, Modlitbová 2018, Modlitbová 2019, Modlitbová 2020a]. Also, our and other groups bioimaging studies were summarized in two extensive review papers [Kaiser 2012, Modlitbová 2020b].

Our last plant study deals with bioimaging of Cd contained in CdTe Quantum Dots (QD) in *Sinapis alba* (white mustard) plant. The LIBS maps with a lateral resolution of 100 μm were constructed for the whole plants, and maps with a lateral resolution of 25 μm (micro-LIBS arrangement) were used to analyze only the most interesting parts of plants with Cd presence (e.g. root tips or a part crossing the root into the above-ground part) [Modlitbová 2020a].

2. EXPERIMENTAL

LIBS system consisting of a nanosecond laser (CFR 400, Quantel, France; 532 nm, 20 Hz, 10 ns), a Czerny-Turner spectrometer Shamrock (Andor, Great Britain), and an ICCD

detector iStar 734 (Andor, Great Britain). For micro-LIBS system, the extensive changes in the focusing and collecting optics were done.

2.1. Plant LIBS experiments

The apparatus settings were optimized a priori: 0.15 μs of the gate delay, 50 μs of the detection integration time, and 20 mJ of the laser pulse energy. The whole plant area was analyzed in a raster of spots with a 100 μm lateral resolution. The emission line Cd I 508.56 nm was selected based on our previous experience. The line intensity was evaluated as the maximum line intensity after the appropriate background subtraction and after the internal standardization to total emissivity. Intensities of analytical emission lines were then depicted as 2D maps representing the spatial distribution of Cd in plant samples.

2.2. Plant micro-LIBS experiments

The apparatus settings were optimized a priori: 0.4 μs of the gate delay, 50 μs of the detection integration time, and 3.0 mJ of the laser pulse energy. The only several small parts per plant were analyzed (5 × 5 mm) in a raster of spots with a 25 μm lateral resolution. The emission line Cd I 361.05 nm was evaluated because it showed better intensity than the previously used emission line Cd I 508.56 nm. However, the same line intensity was evaluated as for LIBS experiments.

3. RESULTS AND DISCUSSION

The two-dimensional maps of Cd distribution in *S. alba* were obtained using LIBS together with micro-LIBS analyses. LIBS with a lateral resolution of 100 μm was used to assess Cd distribution through the whole plant (**Figure 1.** A), while micro-LIBS with a lateral resolution of 25 μm was used to analyse only the most interesting parts of plants, for example root tips or the part crossing the root into the above-ground part (**Figure 1.** B).

The size of LIBS and micro-LIBS maps was chosen based on the assumed time of analysis. The time of LIBS measurement of an average map of the whole plant with 100 μm lateral resolution was around 45 min (laser pulse frequency 20 Hz). It is not possible to map routinely the whole plant with a resolution of 25 μm due to significantly rising time of measurements (approximately 6 h).

Only a very little number of LIBS studies in a similar lateral resolution dealing with NP distribution in plants has been published until now. Our research group successfully presented three papers dealing with distribution of silver NPs, Cd-based QDs, and photon-upconversion NPs in plants [Krajcarová 2017, Modlitbová 2018, Modlitbová 2019]. Until now, the best lateral resolution was achieved by Krajcarová

[Krajcarová 2017], who analyzed silver NPs in cross-sections of *Vicia faba* with lateral resolution 50 µm.

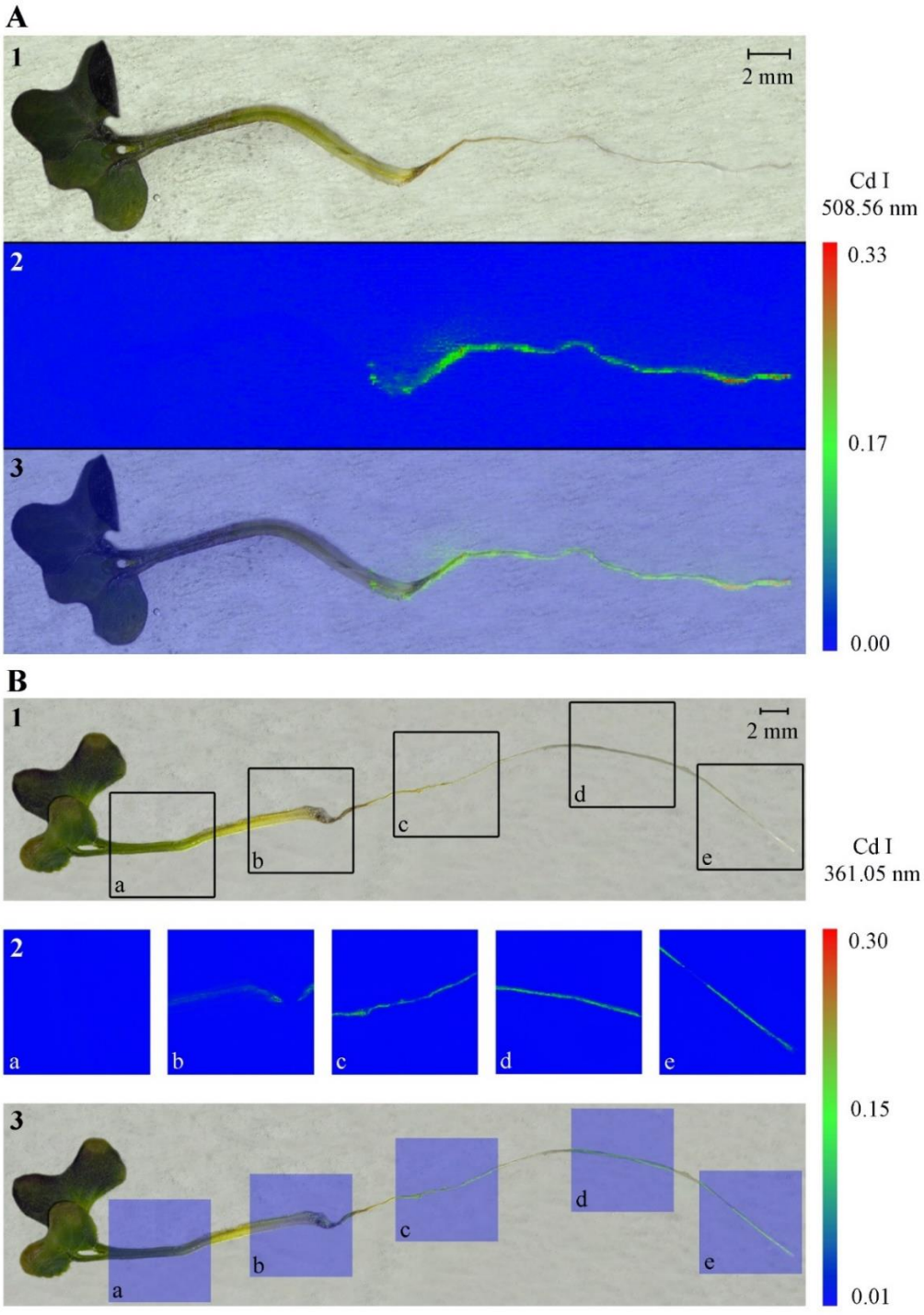

Figure 1. **A)** (1) Photograph of *S. alba* plant exposed to CdTe QDs at the nominal concentration 200 µM Cd before LIBS measurements. (2) LIBS maps constructed for Cd I 508.56 nm. (3) Overlap of the original photograph of the plant with LIBS map. **B)** (1) Photograph of *S. alba* plant exposed to CdTe QDs at the nominal concentration 200 µM Cd before micro-LIBS measurements together with marked spot analyzed by micro-LIBS (a-e). (2) Micro-LIBS maps constructed for Cd I 361.05 nm. (3) Overlap of the original photograph of the plant with micro-LIBS maps. The scale shows the total emissivity of the selected emission lines. [Modlitbová 2020].

4. CONCLUSIONS

The LIBS proved to be a relatively fast analysis with a sufficient precision and an acceptable spatial resolution for plant analysis. Also, the great usefulness of LIBS in the fast mapping of relatively large samples was demonstrated in the present case study while micro-LIBS was shown to be suitable for detail investigations of small selected parts of biological samples.

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