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Aachen, April 7th 2016

Cover letter for invited manuscript

Dear Alison Hamlin,

We would like you to consider the enclosed manuscript, entitled "A rapid laser probing method facilitates the non-invasive and contact-free determination of leaf thermal properties" by Johannes Buyel *et al.*, for publication in *Journal of Visualized Experiments*. We have written this manuscript upon your personal request.

The manuscript describes a contact-free non-destructive near infrared (NIR) laser probing method to determine the specific heat capacity and thermal conductivity of leaves and other solid biological samples. The two thermal parameters are important for the design of equipment used for heat treatment of biological materials as in food and feed applications as well as in molecular pharming. Additionally, the method will be of interest to environmental biologists. We used an approach that requires minimal laboratory equipment, that can be set up quickly and facilitates a high sample throughput with measurement times of three minutes per specimen. The method comprises a two-staged measurement phase. First, the sample is heated up locally by a short laser pulse after which the specific heat capacity of the sample is calculated based on the maximum temperature difference between sample and environment as well as its mass. Next, the temperature profile of the sample is recorded with an infrared sensor for up to one minute. The observed decrease in temperature over time is combined with an energy balance for conduction, convection and thermal radiation to calculate the thermal conductivity of the sample. Hence, both thermal properties, specific heat capacity and thermal conductivity, can be determined in a single rapid measurement which is contact-free and non-destructive. These are important advantages that, to our knowledge, have not been reported for previously described methods. In the protocol section of the manuscript we provide all the required details to prepare the samples, set up the apparatus for measurement, collect the temperature profiles and conduct the calculations for the specific heat capacity and thermal

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conductivity. In the results section we provide data acquired for two plant species, *Nicotiana tabacum* and *N. benthamiana*. We discuss the parameters that have the strongest impact on the measurement and calculations as well as approaches how both can be improved. If solely based on a written description, the protocol may be difficult to reproduce, especially the measurement apparatus. Therefore, visual instructions will greatly facilitate the implementation and success rate of the method when transferred to other laboratories, which is why we believe the manuscript will be of great interest to your readers.

Johannes Buyel contributed the computational work, data, text, figures and scientific knowledge as well as experiment planning and data interpretation in this manuscript. Hannah Gruchow contributed the laboratory work, data interpretation, manuscript writing and figure design. Martin Wehner provided expert knowledge in optics as well as engineering expertise to effectively set up the apparatus. During the submission process, we received assistance from you, Alison Hamlin, Associate Editor - Environment of the *Journal of Visualized Experiments*.

We would like to suggest the following potential reviewers for the manuscript:

Zivko Nikolov - Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX 77843, USA, znikolov@tamu.edu

Eva Stöger - Institute of Applied Genetics and Cell Biology (IAGZ), University of Natural Resources and Life Sciences, Vienna, Austria, eva.stoeger@boku.ac.at

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We look forward to hearing from you in due course.

Yours sincerely,



(Dr. Johannes F. Buyel, Head of Department)