ÚFE Institute of Photonics and Electronics
The Czech Academy of Sciences

Supervisor's review of the doctoral thesis

Title: Ultra-weak photon emission from biological samples

Doctoral student: Mgr. Petra Vahalová

Supervisor: Ing. Michal Cifra, Ph.D.

Petra Vahalová's doctoral thesis centers around an in-depth exploration of specific aspects within the realm of experimental analysis of biological autoluminescence (BAL), often referred to as ultra-weak photon emission. The core of this thesis comprises three articles for which Petra is the primary author, alongside one chapter where she serves as a co-author.

The doctoral thesis starts with a comprehensive overview of existing techniques for monitoring oxidation in biosamples is presented, along with their fundamental characteristics and capabilities (Ch. 1 and 2). The focus then narrows onto one specific method that utilizes BAL, delving deep into the intricate correlations between BAL attributes and selected physical, chemical, and biological parameters which is a subject of the *first (review) paper in the thesis* (Ch. 4) The literature pertaining to the relationships between BAL and various factors influencing the BAL process, such as ROS generation and different oxidative products, is systematically categorized into two groups based on the complexity of the samples. A concise summary of these references is consolidated, encapsulated in Table 2. Furthermore, the potential avenues for amplifying the typically subtle BAL signals are explored and elaborated upon (*book chapter, Appendix A*).

The experimental findings obtained by Petra through her research confirm the involvement of ROS in the mechanistic pathway leading to BAL, substantiating the positive correlation between ROS abundance and BAL intensity (*second and third paper*, chapters 5 and 6). Notably, a novel reaction scheme is proposed within a BSA sample, wherein BAL is significantly augmented through electrogenerated ROS (Ch. 6). Moreover, the thesis advances our understanding of BAL associations with a spectrum of physical, chemical, and biological parameters, encompassing the following realms: 1) spontaneous BAL in yeast *Saccharomyces cerevisiae* cell cultures during their growth in a bioreactor (Ch. 5), 2) chemically enhanced BAL via hydroxyl radicals originating from the Fenton reaction in yeast cell cultures and the protein BSA (Ch. 5 and 6), and 3) physically augmented BAL through PEF in the BSA protein (Ch. 6). These discoveries collectively bolster the potential utility of

BAL as a non-invasive monitoring and diagnostic tool in both medical and food industry domains.

Throughout her research journey, Petra exhibited resilience and determination, contributing to the development of several protocols, experimental procedures, and techniques that are now serving as foundations for the ongoing research efforts of other team members. Beyond her research endeavors, Petra actively participated in various operational roles within our research team at the Institute of Photonics and Electronics—ranging from being the manager of the luminescence lab, guardian of deep freezers, and social coordinator to serving as the first aid contact and participating in various institute committees and other duties. Petra and her colleagues presented her results at several international conferences.

As proved by the results of her thesis, Petra learned to independently solve variety technological and scientific questions. Therefore, I strongly suggest that her thesis is accepted for the defense and that she is awarded the doctoral title.

Prague, 9.8. 2023

Ing. Michal Cifra, Ph.D.