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046 MALDI mass spectrometry imaging: an in situ histoproteomic approach to monitor the response of pollinators to their stressors

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Facing many environmental stress factors, pollinator populations continue to decline precipitously, placing increasing pressure on managed pollinators like honeybees to fulfil crop pollination. Those factors do not operate only individually, but and more often so, in combination with each other. How deeply these stressors (biotic and abiotic; individually or combined) impact honeybee colonies is not well understood and remains a major challenge for the beekeeping industry.

We have been developing a protocol investigating in situ the bee physiological status, by monitoring the protein signatures with respect to bee histology using MALDI Mass Spectrometry Imaging (MSI) or MALDI histoproteomics. MALDI-MSI is a cutting-edge imaging technology that allows to map the distribution of hundreds of biomolecules within organs, tissues and even whole body sections in a single experiment (Arafah et al., 2012; Bulet & Arafah, 2013, 2014a,b; Schwamborn et al., 2016; Lagarrigue et al., 2016). MALDI-MSI does not require labeling biomolecules prior to detection; from its initial biological application for a better understanding of biological organisms, symbiosis and host-response to infection, this technology is now used for biomarker discovery in clinical settings. MALDI-MSI has been applied to study the relationships between host, pathogens and other stressors and to follow some already known immune related peptides within sections of whole bees and on biological features (surface epithelia…) involved in the response to stressors. We have designed and tested an experimental procedure for imaging the molecular fingerprints of both the bee whole body and its isolated digestive tract. Our preliminary data are suggesting that MALDI MSI which result in spatio-temporal images of a physiological status of a whole organism is a useful innovative approach for monitoring the honeybee health. Moreover, we have very recently successfully applied this approach to other pollinators.

047 Assessment of honey bee cells using deep learning

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Temporal assessment of honey bee colony strength is required for different applications in many research projects. This task often requires counting the number of cells with brood and food reserves multiple times a year from images taken in the apiary. There are thousands of cells in each frame, which makes manual counting a time-consuming and tedious activity. Thus, the assessment of frames has been frequently been performed in the apiary in an approximate way by using methods such as the Liebefeld. The automation of this process using modern imaging processing techniques represents a major advance. The objective of this work was to develop a software capable of extracting each cell from frame images, classify its content and display the results to the researcher in a simple way. The cells' contents display a high variation of patterns which added to light variation make their classification by software a challenging endeavor. To address this challenge, we used Deep Neural Networks (DNNs) for image processing. DNNs are known by achieving the state-of-art in many fields of study including image classification, because they can learn features that best describe the content being classified, such as the interior of frame cells. Our DNN model was trained with over 60,000 manually labeled images whose cells were classified into seven classes: egg, larvae, capped larvae, honey, nectar, pollen, and empty. Our contribution is an end-to-end software capable of doing automatic background removal, cell detection, and classification of its content based on an input image. With this software the researcher is able to achieve an average accuracy of 94% over all classes and get better results compared with approximation methods and previous techniques that used handmade features like color and texture.

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