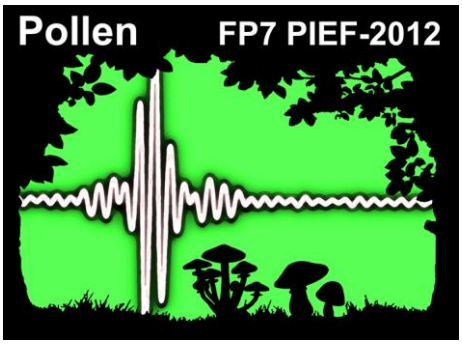
Final Report for PIEF-GA-2012-328289

FP7-PEOPLE-2012 Intra-European Fellowships:
Pollen – *Plant Phenotyping by Vibrational Spectroscopy of Pollen*



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Final Publishable Summary Report:

POLLEN project has developed and standardised vibrational spectroscopy for identification and biochemical characterization of plant pollen. In addition to analysis of pollen, the project has established the same methodologies in analyses of plant and fungal spores. The original methodologies developed in the project have enabled objective identification and comprehensive characterisation of pollen and spores. A novel spectroscopy-based approach for screening, monitoring and analysis of these microorganisms has the potential to expand current knowledge and research, and to advance industrial and social applications by providing new insights on plants and fungi.

Pollen and spores are abundant and widespread in all sorts of environments, from terrestrial, aquatic and atmospheric ecosystems, all the way to urban and indoor environments. Pollen and spores are the reproductive structures (microorganisms) of plants and fungi, and thus have key function during plant and fungal life cycles. In the form of microfossils they are present in all types of soil and rock sediments, and thus their identification is vital for the reconstruction of past environments and for the understanding of the causes of environmental changes. Therefore, monitoring of these microorganisms is important for management of environment, including pollution, biodiversity and climate impact on wild and cultivated communities (including agronomy and forestry). Moreover, monitoring is vital due to their, often harmful, impact on society, by causing diseases, food spoilage and material decomposition. For example, aerollergenic pollens and spores trigger respiratory diseases in susceptible individuals that are affecting 20% of European population (with the increased prevalence), causing multibillion costs of medical treatments and reduced work productivity. For the last century, palynology (the science of pollen and spores) has been limited by laborious, slow and costly methods. By implementing spectroscopy-based approach, POLLEN project has created a paradigm shift that will advance palynology into new scientific and applied fields.

The research, training and dissemination activities of POLLEN project have been carried without major deviations from the schedule, and all critical objectives have been achieved during the project. Moreover, due to acquired additional funding some planned activities have been modified and expanded.

The first activity, '*Spectral pre-processing and development of standardised database of pollen spectra*' involved a wider research community in Europe in the establishment of the database as originally expected. At the beginning of the project, we initialized a collaborative research with group of Dr. Janina Kneipp at the Humboldt University of Berlin. The collaborative research has been further financed by the Research Council of Norway (RCN project Nº. 233941) and German Academic Exchange Service (DAAD): '*Combined FTIR and Raman analysis of pollen composition for studying plant adaptation to environmental changes*'. The collaboration addressed standardisation of sampling techniques for FTIR and Raman spectroscopy of pollen, which is the main challenge for broader application of these methods by the scientific community. The collaboration yielded the design of a standardized database of pollen vibrational spectra, which will represent the raw material for a wide range of botanical research, from agronomy to ecology. The database will be published in short time. A new method for obtaining the pure absorbance spectra of biological systems has been developed, and published in a peer review paper '*Recovery of absorbance spectra of micrometer-sized biological and inanimate particles*' (*Analyst*, 140 (**2015**) 3273).

The second activity, '*Collection of pollen samples and recording of their vibrational spectra*', covered outdoor sampling of pollen and spores across Europe, from Northern Mediterranean to Arctic, as well as two greenhouse experiments at NMBU. In total, during the project timeframe, more than 800 outdoor and more than 900 indoor samples have been collected. Moreover, through the collaborative research with Dr. Maarten Christenhusz, we have initiated studies on non-flowering vascular plants: ferns and fern allies. The studies (initiated in May 2015) have covered more than 100 species of plant spores to date, with more samples planned for the future. This activity was further expended by new measurement methodologies that were developed and published in a peer review paper '*Vibrational microspectroscopy enables chemical characterization of single pollen grains as well as comparative analysis of plant species based on pollen ultrastructure*' (*Planta*, 242 (2015) 1237).

The third activity, '*Classification of pollen spectral data and biochemical characterization of pollen*', has resulted with detailed biochemical characterizations of plant pollen and fungal spores. The

results were published in a peer review papers '*Characterizing Aeroallergens by Infrared Spectroscopy of Fungal Spores and Pollen*' (*PLOS One*, 10 (2015) e0124240.) and '*A multiscale vibrational spectroscopic approach for identification and biochemical characterization of pollen*' (*PLOS One*, 10 (2015) e0137899). One of the promising goals of the project is the development of a method for aerobiological monitoring of pollen based on vibrational spectroscopy. We have decided to work on the development of this method during the project timeframe due to the significant progress in the standardisation of a high-throughput vibrational spectroscopic technique for measurement of pollen. The financing for the development of the method was obtained by NMBU, Forskningsavdelingen, under the program 'Idekonkurransen for forskere 2013' (project Nº. 1301026403): '*Automatic data collection and processing system for monitoring of aeroallergen bioparticles*'. The goal of this project is to develop a system for cost-efficient gathering of information on allergenic air quality. Preliminary studies were successful, and one peer review paper with title '*Analysis of allergenic pollen by FTIR microspectroscopy*' was submitted and reviewed positively in *Analytical Chemistry* (minor revision pending). In this regard, we have initiated research collaboration with the Center of Allergy and Environment (ZAUM) at the Technical University of Munich. The group of Prof. Jeroen Buters at ZAUM has experience on developing forecasting methods which are important for the prevention and the treatment of allergies.

The fourth activity, '*Correlation analysis of pollen spectral data and climate data*', has been accomplished through two greenhouse studies conducted at NMBU. In addition, a large collection of Arctic plant samples from Svalbard has been obtained through collaborative research with Dr. Pernille Bronken Eidesen at the University Centre in Svalbard. The research has been financed by Svalbard Science Forum as a personal research grant for the fellow, Dr. Boris Zimmermann (AFG project Nº. 246125): '*Phenotyping of flora of Svalbard by vibrational spectroscopy of pollen and seeds*'. Svalbard is an isolated archipelago characterized by an arctic climate, permafrost and scarce vegetation adapted to extreme conditions. Thus, it presents a perfect experimental pool for investigation of reproduction fitness and strategies in the extreme environment and for providing valuable new information on climate-related plant processes. Moreover, the aforementioned collaboration with the Humboldt University of Berlin relies on transnational research cooperation in order to gather relevant data on plant-environment interactions.

The last activity, '*Training*', and the interdisciplinary nature of pollen research has contributed to the full development of skills, expertise, and competence of the fellow scientist. Dr. Zimmermann has attended regular courses at the NMBU, and has conducted training and the experimental work at biospectroscopy facilities at Campus Ås. The fellow has cooperated closely with PhD student Murat Bağcıoğlu as well as master students Kjersti Misfjord and Maria Høegh Berdahl. All students have been involved in pollen research, and the results of their cooperation with Dr. Zimmermann will be published as master and PhD thesis (master thesis are already available on-line on NMBU webpages). The fellow has published the research results in four peer review papers, published in high-impact journals (Q1) as defined by Web of Science JCR and Scopus SCI. Another three peer review papers with work titles '*Pollen phenotyping by vibrational spectroscopy*', '*High-throughput FTIR characterization of pollen for plant phenotyping*' and '*A greenhouse study for assessing climate effects on pollen composition by FTIR spectroscopy*' are in preparation, with tentative publication dates in the first half of 2016. Moreover, the project results were presented at seven conferences and workshops. Finally, the fellow has presented POLLEN project to general public and to the broad scientific community in Norway (the Norwegian Institute of Public Health, Campus Ås and the University of Bergen), Croatia (the University of Zagreb), Germany (the Technical University of Munich), and the UK (the Royal Botanic Gardens, Kew).