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Review Article

Integration of Aerosol Spectrometry Techniques with Other Methods for Spore Concentration Monitoring for Analysis of Degradation of Wooden Structures: from Lens-Less Flow Morphometry to Aerosol Mass Spectrometry and in Situ Elemental Microanalysis

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Abstract

It is well known that any pathogenic fungi or mold play a significant role in the degradation of wooden structures, such as timber framings, fachwerk houses and natural furniture. They have the ability to break down cellulose and hemicellulose, which are key components of wood, leading to decay and mechanical / structural weakness. As they colonize wooden surfaces, they can cause discoloration, warping, and ultimately compromise the structural integrity of the material. In addition to wood, pathogenic fungi and mold can also affect a variety of other ma-terials, including textiles, paper, and even certain types of plastics. Con-sequently analyzing the types of mold and the morphology of fungal spores is crucial in combating fungal damage to wooden materials. Dif-ferent species of fungi have varying spore morphologies, growth pat-terns and destructive capabilities, making it essential to identify them accurately for effective treatment and prevention strategies. Aerosol measurements and spectroscopy offer promising avenues for the detec-tion of fungal spores and mold in airborne streams. By leveraging aero-sol measurement techniques, such as particle counters and optical parti-cle sizers, it becomes feasible to quantify the concentration of fungal spores within an air stream. These methods enable the real-time moni-toring of airborne spore levels, providing valuable insights into the dy-namics of fungal dispersion. By examining the unique spectral and morphometric signatures associated with fungal spores, it becomes pos-sible to differentiate them from other airborne particles, thus facilitating their identification within complex aerosol mixtures. Integrating aerosol measurements with spectroscopic analysis holds significant potential for advancing the rapid and accurate identification of fungal spores and mold in air streams. This combined approach not only enhances our un-derstanding of airborne fungal dynamics but also offers practical impli-cations for indoor air quality assessment, environmental monitoring and wood engineering damage prevention.

Keywords: Cellulose and Hemicellulose, Mold Degradation of Wooden Structure, Timber Framing, Fachwerk Houses, Furniture Materials, Spores, Aero-sol Analysis, Aerosol Mass Spectrometry, DAS; ICP MS, Lens-Less Instrumentation, 2D FFT, Morphometry

1. Aerosol Counters

The role of fungal spores in the formation of biogenic aerosols, includ-ing those that enter the human homes, and hence, are directly related to sanitation and hygiene problems, is well known [1,2]. Analyzing the types of mold and the morphology of fungal spores is crucial in combat-ing fungal damage to wooden materials. Different species of fungi have varying growth patterns and destructive capabilities, making it essential to identify them accurately for effective treatment and prevention strat-egies. Therefore, it seems logical to introduce aerosol control methods with pattern recognition in monitoring indoor air for the purposes of medical mycology. At the same time, it is obviously impossible to per-form online monitoring and counting of the individual types of spores using conventional laboratory methods such as cytometry and fluores-cent sorting using specific labels. Hence, it is necessary to look for the new methods of analysis, explicating them from the physics of aerosol systems without any chemical modification and introduction of fluores-cent labels (the so-called label-free analysis).

From the classical works of the team of academician I.V. Sokolov-Petryanov on aerosols, which resulted in the development of industrial diffusion aerosol spectrometers, as well as the automata used in the analysis of cloudiness in the Venus atmosphere, the main principles of constructing optical systems for aerosol disperse system control / moni-toring with different registration angles are known [3-7]. These control schemes are still used in the 21st century [8,9]. Examples of diffusion aerosol spectrometers and optical particle counters (based on I.V. Sokolov-Petryanov and Yu.V. Zhulanov ideas) engineered by P.Yu. Makaveev provided at Figures 1, 2.

Such installations are superior in angular characteristics to the conven-tional cytometers: "since the type of function ... also depends on the angle at which the scattered light is registered and on the receiver aper-ture," it is possible to improve "the sensitivity of the pulse amplitude to the refractive index ... for the scattered light registration angles of 75-105° and 31-54°"… "for the particles with a size of 0.5 microns and above." At the same time, "... favorable conditions for separate registra-tion are observed at 31-54° angles" [7]. That is, using a scheme with a tunable angle, operating on the principles of microrefractometric analy-sis, it is possible to obtain more information about the habitus of spores than using standard cytometers with fluorochrome staining of the sam-ple, since standard flow cytometers detect only: a) light scattering at small angles (from 1° to 10°); b) light scattering at an angle of 90°; since all the information is captured through a number of fluorescent channels (from 2 to 20, which requires the use of an appropriate range of expen-sive dyes). Conventional aerosol methods with different control angles also do not provide sufficiently complete information about the particle habitus, which is necessary for solving identification problems in quan-titative sporometry. Therefore, a complementary method for monitoring the spore habit at the input of the aerosol counter or in the measuring cell itself is necessary (in some special cases, in situ analysis in the filter plane is also possible, but we do not consider here this approach).

2. Lens-Less Instruments

One of the most up-to-date methods for aerosol monitoring is lensless (including holographic) microscopy (that is, by definition, multi-angle according to the measurement scheme) [10,11]. It is also used for the analysis of bioaerosols [12,13]. Lensless cytometers operate on the same principle of holographic registration [14-17]. Such devices can also be used for the analysis of fungi (for example, yeast) and their spores (for example, for microsporidia [18,19]. When using pattern recognition and machine learning technologies, which make it possible to identify mi-crostructures on a chip or in a projection cell, this method can be used as a reference one or a validating tool for the species or taxonomic iden-tification of spores at the input of an aerosol counter. Calibration of such equipment with the real spores can be carried out on the installa-tions with aerosol spore generators [20-24].

A significant problem of modern environmental monitoring is continu-ous analysis of the emission of fungal spores spreading in an aerosol form [25,26]. This problem is usually solved by fluorescent label-based measurements, as well as by the automated microscopic methods (often also fluorescent ones) with further processing of the video stream and identifying the number of spores of a given taxonomy using contour analysis (for example, Sobel-Feldman operator, etc.) or texture analysis (for example, bit maps of entropy, contrast, energy at different rotation angles) [27,28]. Aerodynamic measurements are carried out less fre-quently, and their results strongly depend on humidity, which correlates with the spore viability, since for many types of spores "water content" or shell hydration / the presence of bound water in the peripheral layers of the cell wall directly correlate with it. Therefore, analyzers of the aerosol forms of spores can be integrated with the liquid counters, mak-ing them more universal in terms of the possible habitats / sample loca-tions [29,30]. This coincides well with the idea of aerohydrodynamic liquid and aerosol cytometry based on specialized universal counters [31].

3. From (micro)structural to Chemical Identification of Species

However, this line of work cannot be considered complete without identifying "chemistry," that is, the spore chemical composition and correlation dependence between the parameters of the spores detected and the conditions of their existence. Thus, in the penultimate cited work, the contribution of the spores to the atmospheric aerosol balance is considered, taking into account both the contribution of intrinsic car-bohydrates and the contribution of inorganic ions, and in the contribu-tion of water and precipitation mechanisms to the effectiveness of the fungal spore contribution to the content of organic carbon in atmos-phere (theoretically, to a certain extent, extrapolated to the substrato-spheric space as a whole) [32]. So, as a component of bioaerosols, spores can influence atmospheric chemistry, carbon balance and (micro-) climate. They can also serve as condensation centers for the atmospher-ic moisture, participating in differential sedimentation, and move along with the sea and freshwater aerosols, which means that it is reasonable to apply for them the corresponding methods of analysis of such sys-tems during condensation, sedimentation, and electrostatic mobility of droplet-dispersed aerosols under natural hydrological conditions, ex-trapolated from physics of aerodisperse systems [33-37]. They, accord-ing to, provide the opportunity to "analyze aerodisperse systems by physical and chemical composition", since "simultaneous measurement of the amplitude and size ... of particles ... provides the opportunity for classification" (of spores) "... according to their refractive index, [that is] by physical and chemical composition." However, direct biochemical / microanalytical identification of the spore composition cannot be ob-tained in this way. Because of this, it is necessary to instrumentally in-tegrate the methods of aerodisperse analysis based on refractometric principles with the other methods of chemical composition analysis when pumping atmospheric air through a single apparatus.

Figure 1: A Collage of Different Modes of Analytical Visualization and Particle Countiong and Size Analysis in Old-Type Diffusion Aero-sol Spectrometers and Aerosol Counters Developed by Yu.V. Zhulanov and P.Yu. Makaveev in NIFKHI (before the Closing of Laboratory and NIFKHI Itself in 2019-2020)

4. From ICP MS and Aerosol Mass Spectrometry to Spore MS Imaging

We propose the integration of aerosol spectrometric sporometry tech-niques applicable in real environments, in situ, with a number of well-known analytical techniques. Within the framework of the approach of integrating methods based on light scattering and mass spectrometry associated with sampling from the aerosol phase [38], it is proposed to control in real time (simultaneously with the size measuring of the aero-sol particles) organic constituents in the chemical composition of spores (and other aerosol particles similar insize) [39,40]. In particular, it seems possible to use laser ionization techniques [41,42]. To analyze the ele-mental and isotopic composition (different isotopes of chemical ele-ments cause broadening and even complete splitting of some spectral lines), it is proposed to use, in addition to ICP-MS, methods of atomic emission or atomic absorption spectrometry with direct sample injection (as in aerosol MS [43]) with either a wavelength sweep or at fixed wavelengths to identify individual chemical elements [44,45]. The ap-plication of in situ MS imaging techniques for single particle analysis can also be useful. It should be noted that the optimality of the optical methods of MS ionization, as well as the applicability of a number of optical spectrometry methods in the case of spore analyzers based on the optical light sources, can be determined by the multiplexability of all (or a significant part) of such methods using optical counting meth-ods and analysis of the properties of the spore particles, including ma-chine learning for the analysis of spores using reference samples [42].

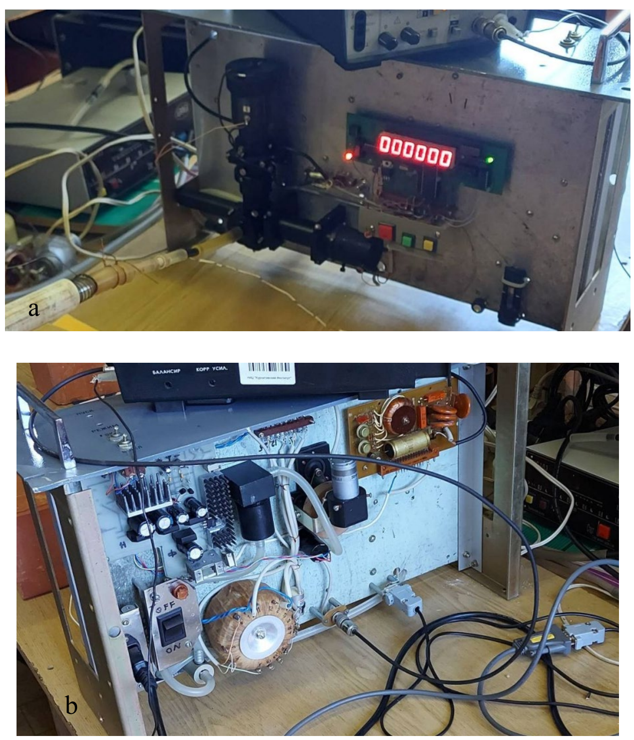

Figure 2: Experimental Setup for Spore Aerosol Measurement Devel-oped by P.Yu. Makaveev in the Framework of O.V. Gradov's Ideology Described in [37,46,47].

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