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Letter to Editor

Direct Implementation of Physical Principles and Laser Measurement Technologies from Optics of Dispersed Systems to Medical Mycology with Taxonomic Identification of Samples by Spectra of Spore Sizes and Morphologies

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The use of cytometry techniques for spore analysis has been well established since the late 1980s and early 1990s. [1], and already since the beginning of the 1990s. To analyze the species of spores, progressive multidimensional clustering algorithms and expert systems and neural networks were used (for example, when identifying spores of asco- and basidiomycetes [2,3]). Techniques used to identify and sort spores were typically based on FACS approaches, which required sample staining. This requirement is, in most cases (excluding the so-called imaging cytometry with video stream analysis) still relevant today [4]. Because of this, the emphasis is not on analyzing the shape and physical properties of spores, but on the content of substances in them that are stained with dyes/labels that are different in selectivity. Particularly noteworthy are ratiometric staining methods and fluorescence in situ hybridization (FISH). As a result, over the past three decades, many works have been published on the analysis of the content of spore components, such as DNA (up to the full genome scale [5]) and proteins [6,7]. But on the physical and geometric properties of spores, as a rule, only works on "imaging" cytometry are published, and a number of methods - such as scanning flow cytometry - have not actually been used to analyze mycological objects as such. The lack of information about the geometry of spores can be eliminated by combining flow cytometry and scanning electron microscopy, including immunoelectron microscopy [8,9], but in most cases (excluding exotic ESEM techniques - scanning electron microscopy with a programmable environment [10]) it leads to irreversible dehydration-denaturation of the cytoplasm during evacuation of the SEM column and sample preparation (with sputtering, as a rule, on a vacuum post or ion plasma source of Ag, Pt, Pt-Pd, etc.). As follows from the above, direct in situ analysis of spore content in the atmosphere is impossible in conventional flow cytometry. Considering that the size of fungal spores ranges from a few to more than a hundred microns, in principle, it is extremely difficult to create an effective focusing system for a spore sorter. Due to the significant difference in the size and mass of spores, both the acoustic and acoustofluidic scheme, as well as the electrostatic scheme, commonly used [11], may actually be ineffective. This puts the optimization of cytometry parameters at the forefront - not only in terms of identification descriptors, but also in the instrumental implementation of sorting schemes / technical processes [12], which

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is a problem of non-differentiated optimization or search for local optima. If the species sample varies during sorting and continuous sampling (which inevitably occurs when sampling from the atmosphere without preliminary filtration), then the parameters of the adaptive device / hardware and software complex for spore analysis will also vary, which, all other things being equal, will inevitably be a source of difficulties in data processing and interpretation, which will need to be carried out taking into account statistical metadata (as in photon correlation spectrometers such as zeta sizers). Meanwhile, sampling from the atmosphere is a self-evidently obvious solution if we are talking, as usual in aerobiology and atmospheric ecology, about analyzing spores in situ / directly in biogeocenotic conditions, which are characteristic of a given complex of species, known ecological niches [13,14]. Therefore, we propose the introduction of direct methods for flow-through aerosol-optical analysis of spores, based on classical methods and technologies for the analysis of aerodisperse systems, created under the leadership of Academician of the USSR Academy of Sciences I.V. Sokolov-Petryanov [15] and used up to the analysis of cosmic, [exo-]planetary aerosols ("astrosols"), in particular on Venus [16]. This approach, in particular, allows not only to separate the composition of spore aerosols, but also to analyze their physical and chemical composition (with certain instrumental refinement and modification). According to the classic work [15], "... opens up the possibility of not only ... dispersed analysis of aerosols, but also the analysis of aerodispersed systems by physical and chemical composition," "simultaneous measurement of the amplitude and size ... of particles ... creates the opportunity for classification them according to their refractive index, [that is] according to their physical and chemical composition." At the same time, the sizes of biological particles studied at installations, the ideology of which was laid down under Academician Sokolov-Petryanov, can vary significantly (from viruses to blastomeres [17-19]). This allows you not to change, unlike many laboratory cytometers, the settings of a robust field device / mobile hardware and software complex, providing an objective and stable analysis of spores. In contrast to classical flow cytometers, which are flow cytofluorimeters and flow cytometers (in the words of Prof. V.P. Maltsev, the author of the method of scanning flow cytometry from the SB RAS), "refractometric analyzers of the intrinsic properties of spores" based on the above principle are strict (calibrated according to latexes and dye-state-independent) metrological/robust monitoring instruments, which can be recommended to a mycologist who does not have sufficient facilities and competencies for molecular analysis to work in the field conditions.

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