Alternatives to manual spore counting for the detection and analysis of airborne fungal spores

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Abstract

We describe three methods to detect and identify fungal spores in environmental samples using microscopy and spectroscopy. All methods offer automated sample analysis and represent significant reductions in the time and labor required for sample analysis.

First, a Raman micro-spectroscopy technique was developed to identify spores using their distinct spectral signatures with the goal of developing a robust, automated method. Method development steps included 1) selection of an optimal sampling substrate, 2) acquisition of Raman spectral signatures for micro- and macro-fungi, and 3) automation of library searches.

Second, two automated, image-analysis-based methods were evaluated by comparing them to human sample examination. Digital images of spores were analyzed with a program that automatically measured particle Feret's diameter, area, perimeter, and circularity, which were used to identify single spores and spore clusters.

Third, a computer program was written to rapidly recognize unusual spore concentrations through identification of near monodisperse particle distributions in the respirable size range. Its feasibility was evaluated using real and simulated data.

Relevance

Easy and accurate identification of fungal spores is relevant to many aspects of human health and comfort. Light microscopy is a widely used and requires little sample preparation. However, major disadvantages are its inability to distinguish viable from non-viable cells and dependence on a human analyst's ability to distinguish particles and accurately count and size them.

Raman spectroscopy-based detection of biological materials offers a complementary approach to the traditional methods of spore identification. Automated image analysis of impactor samples may be more accurate and faster than manual examination if sequential samples could be fed directly into an image acquisition and data analysis device. We anticipate potential application of these approaches in many fields including public health, biological defense, ecology, environmental microbiology, agriculture, and forensics.

Uncertainty

Ability of Raman microspectroscopy to distinguish fungal species may be limited to the genus level. An automated system incorrectly may classify image artifacts as particles if the artifacts meet the program's recognition criteria. Automated detection of unusual spore concentrations assumes that the aerosol would consist of large numbers of uniformly sized, nearly spherical particles.