ABSTRACT BOOK

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# SCIENCE GOALS AND EXPERIMENTS

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SPACE RESEARCH INSTITUTE (IKI)
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# A CONCEPT OF A PROBE FOR PARTICLE ANALYSIS AND LIFE DETECTION IN ICY ENVIRONMENTS

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**Introduction:**

Reliable methods for the rapid in-situ detection, quantification and identification of microscopic organisms in water and glacial environments are of major importance in many research areas from microbial biotechnology and biosafety to environmental monitoring and astrobiological space exploration.

Natural water habitats typically consist of many different microbial groups. The detection and identification of bacteria in such highly heterogeneous suspensions is a real challenge for in-situ real-time analysis. Nowadays, the common technique for biological characterization is probe sampling and subsequent culturing. This method has many obvious drawbacks such as necessity of taking a big amount of probes and keeping them in appropriate conditions before and during analysis, long waiting times, difficulties or impossibility to culture some bacterial, fungal and algal species in the laboratory, etc. According to existing reports, the density of microbial cells in ice is in average $10^4$-$10^7$ cells per cm³ with approximately 99% of the cells not cultivable using traditional microbiological methods.

Driven by environmental, occupational, and methodological concerns, there has been growing interest in methods that offer the potential of analyzing and characterizing water-borne particles in-situ and in real-time. Of particular interest would be to differentiate between different kinds of particles. Ideally, biological particles should then be differentiated into individual species. We are working on new concepts and approaches detecting and measuring the microorganisms without the drawbacks of currently available biochemical methods that tend to be destructive, slow and laborious.

We propose a concept for the development and construction of a melting probe equipped with quick and accurate reagentless autofluorescence detection systems combined with light-scattering microsystems and, optionally, microarray chips integrated into the melting probe. This combination of methods may provide sensitivity high enough for detecting very low levels (~10-100 cells/cm$^3$) of microbes within seconds. In the proposed setup, water from melted ice is aspirated into the device and is pre-analyzed in a laser light gate, determining particle properties like size; shape, biological/non-biological nature, live/dead analysis, etc. This is followed by a either fluorescence or FET (field-effect transistor)-based microarray which contains individual array units stamped with various antibodies for specific detection of antigenic determinants. For example, reliable biofilm detection can be accomplished by detecting biomarker chemical species unique to biofilms. Some biofilm markers are tryptophan and exopolysaccharides (EPS), which indicate the presence of living bacteria. Specific antigen-antibody interaction will be further transformed into a fluorescence- or a FET signal, respectively, denoted as a "recognition event", and further analyzed using software that implements neural networks.

**Detection of Intrinsic Autofluorescence of Living Organisms**

Fluorescent microscopy in combination with a light-scattering approach aims at detecting microorganisms based on their intrinsic fluorescence. Essentially, we determine characteristic parameters of fluorescence spectral profiles after specific excitations. This approach is expected to be viability-sensitive and should be able to detect and identify single cells as well as other particles.

Autofluorescence in biological tissues is a common and useful phenomenon arising from a variety of endogenous biomolecules that absorb light in many regions of the near-ultraviolet and visible light spectrum. All living cells have some intrinsic level of autofluorescence, which is most commonly caused by NADH, riboflavins, porphyrins, and flavin coenzymes. One of the primary contributors of plant autofluorescence is chlorophyll, though lignins, carotenes, and xanthophylls also produce a significant level of fluorescence emission when stimulated with the proper wavelengths.

These molecules are excitable over a broad range of wavelengths including the blue region of the spectrum. The emission wavelengths of these autofluorescent molecules when excited in the blue is broad (500–700 nm) and overlaps emission spectra of commonly used fluorescent dyes. The peak autofluorescence emission after 488 nm excitation is in the green region of the spectrum.

Fluorescence methods such as flow-cytometry have long been a favored choice for particle-counting and

size-monitoring as they offer close to real-time response and operate continuously. Two further aspects, namely sensitivity and specificity must be addressed. For the discrimination of biological particles, their intrinsic fluorescence offers significant potential, and several systems have been developed, that incorporate particle fluorescence measurement in conjunction with the measurement of other particle parameters in order to optimize cell discrimination.

In 1995, Pinnick *et al*. described a laser-based particle counter that detected fluorescence and elastic scattering from individual airborne particles as they traversed the beam from a 488-nm argon-ion laser. The low levels of intrinsic fluorescence observed from kaolin, hematite, and polystyrene particles in comparison with that observed from several types of biological particles suggested that the instrument would be useful in discriminating biological from non-biological particles. In an effort to enhance particle discrimination, the same researchers went on to extend the capabilities of the system to record the spectrum of fluorescence from a particle rather than simply the fluorescence magnitude.

More recently this approach has been further developed using two UV excitation frequencies in place of the 488-nm beam. The fluorescence spectra obtained from individual 2–5-mm biological aerosol particles excited by either 266- or 351-nm radiation from a *Q*-switched laser Nd:YAG and Nd:YLF, respectively, illustrated the differences in fluorescence spectra obtained from different biological particles.

Several different fluorescence spectra can be recorded simultaneously. L. Leblanc and E. Dufour performed experiments in which excitation at 250 nm (aromatic amino acids+nucleic acids), 270 nm (tryptophan residues) and 316 nm (NADH) has been used for 25 strains of bacteria in dilute suspensions. Evaluation of the spectra using principal component analysis and hierarchical clustering showed a good reproducibility from culture to culture and a good discrimination of the bacteria. Applying the method of Mahalanobis distances to the spectra would enable the classification and validation of microbial groups. Moreover, advanced signal processing algorithms would allow individual quantification of various bacterial/fungal groups and calculation of heterogeneity (biological diversity) index of the natural suspensions.

Quick and accurate detection of microbial contamination is possible by a combination of fluorescence technologies. H. Mason and co-workers have reported that microbe capture chips, used with a prototype fluorescence detector, are capable of statistically sampling the environment for pathogens (including spores), identifying the specific pathogens/exotoxins, and determining cell viability where appropriate. The technology is sensitive enough to detect very low levels (approximately 20 cells/ $cm^3$ of microbes in seconds).

**Light Scattering-Based Microbial Detection**

In case of lower numbers of cells, their identification can be a serious problem because of the low intensity of optical signals. Any monitoring system must aim for a minimum level of false positives. In an attempt to further reduce the occurrence of false positives, such as may occur when non-biological particles are present with similar size and fluorescence signature to biological particles, additional characteristics must be determined from the scattering particle. One method of achieving this is to examine the spatial pattern of light scattered elastically from the particle, from which both particle size and shape information can be deduced.

Light-scattering-based instruments such as optical particle counters have long been a favored choice for suspended particle count and size monitoring as they offer near-real-time response and can operate continuously without the need for reagents, etc.

The manner in which a particle spatially scatters incident light is a complex function of the size, shape, structure, and orientation of the particle, as well as of the properties of the illuminating radiation wavelength, such as polarization state. With suitable control of some of these variables it is possible to determine parameters related to the shape and structure of the scatterer. This spatial light-scattering analysis, also known as two-dimensional angular optical scattering, has therefore been attracting considerable attention. Dick *et al*. have used multi-angle azimuthal measurements to determine spherical and non-spherical fractions of different systems of particles.

The potential of spatial light scattering analysis can be exploited for particle shape characterization, and the research in this field will result in a number of real-time monitoring systems for application in, for example, particle detection and characterization in water systems.

Summarizing, the very attractive approach would be to create an instrument that similarly records simultaneously an estimate of particle size based on elastic scatter together with intrinsic particle fluorescence detection. Existing successful attempts to produce plots of fluorescent intensity against a scattered light signal in laboratory has illustrated the potential of such dual-parameter measurements in the achievement of precise discrimination of living microorganisms.

In our opinion, a melting probe equipped with autofluorescence-based detection system combined with a light scattering unit, and, optionally, with a microarray chip would be ideally suited to probe icy environments like Europa’s ice layer as well as the polar ice layers of Earth and Mars for recent and extinct live.