Petra Perner
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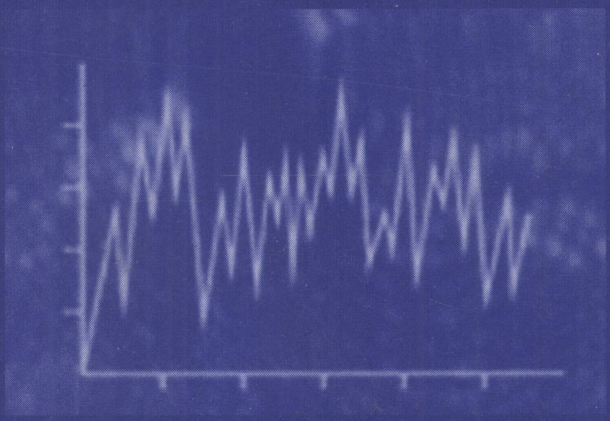
Advances in Mass Data Analysis of Signals and Images in Medicine Biotechnology and Chemistry

International Conferences MDA 2006/2007
Leipzig, Germany, July 2007
Selected Papers



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Advances in Mass Data Analysis of Signals and Images in Medicine Biotechnology and Chemistry

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Lecture Notes in Artificial Intelligence 4826

Edited by J. G. Carbonell and J. Siekmann

Subseries of Lecture Notes in Computer Science

Preface

The automatic analysis of images and signals in medicine, biotechnology, and chemistry is a challenging and demanding field.

Signal-producing procedures by microscopes, spectrometers, and other sensors have found their way into wide fields of medicine, biotechnology, economy, and environmental analysis. With this arises the problem of the automatic mass analysis of signal information. Signal-interpreting systems which generate automatically the desired target statements from the signals are therefore of compelling necessity. The continuation of mass analyses on the basis of classical procedures leads to investments of proportions that are not feasible. New procedures and system architectures are therefore required.

The scope of the International Conference on Mass Data Analysis of Images and Signals in Medicine, Biotechnology and Chemistry MDA (www.mda-signals.de) is to bring together researchers, practitioners, and industry people who are dealing with mass analysis of images and signals to present and discuss recent research in these fields.

The goals of this workshop are to:

- Provide a forum for identifying important contributions and opportunities for research on mass data analysis on microscopic images
- Promote the systematic study of how to apply automatic image analysis and interpretation procedures to that field
- Show case applications of mass data analysis in biology, medicine, and chemistry

Topics of interest include (but are not limited to):

- Techniques and developments of signal and image producing procedures
- Object matching and object tracking in microscopic and video microscopic images
- 1D, 2D, and 3D shape analysis and description
- 1D, 2D, and 3D feature extraction of texture, structure, and location
- Algorithms for 1D, 2D, and 3D signal analysis and interpretation
- Image segmentation algorithms
- Parallelization of image analysis and interpretation algorithms
- Semantic tagging of images from life science applications
- Applications in medicine, biotechnology, chemistry, and others
- Applications in crystallography
- Applications in proteomics
- Applications in 2D and 3D cell images analysis
- Image acquisition procedures for mass data analysis

This volume is a post-proceedings of papers from MDA 2006 and MDA 2007. A large number of the papers propose new image-segmentation techniques for biological and medical applications. Image segmentation is a crucial step in image processing and the accuracy of this step heavily influences the final result. In the methodology the

authors use they try to identify classes of images first and then they propose algorithms that should work robustly and accurate enough for this class of images.

The second portion of papers deals with new applications where imaging and signal-interpretation methods are used. These imaging methods range from optical methods to ultra-sonic microscopy. The applications are in air monitoring for hazardous materials, quality control of cereals, proteomics and drug design, as well as in the characterization of piezo-electric properties. Spectrometers are used for algae classification.

Other papers deal with specific topics such as semantic tagging of biological images, shape characterization under time-varying conditions, and statistical analysis of time-series for DNA sequencing.

Altogether, we were pleased to see how many different problems for imaging and signal interpretation have been presented, showing that there is a tremendous need for automatic methods. We hope we have managed to bring these problems into the center of attention and inspire many other researchers to work on these real applications.

The next International Conferences on Mass Data Analysis of Signals and Images will be held in July 2008. We are looking forward to your submissions.

July 2007

Petra Perner
Ovidio Salvetti

International Conference on Mass Data Analysis of Images and Signals in Medicine, Biotechnology and Chemistry

MDA 2007 / 2006

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Table of Contents

Image Acquisition and Analysis of Hazardous Biological Material in Air

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Abstract. Human beings are exposed every day to bio-aerosols in the various fields of their personal and/or professional daily life. The European Commission has rules protecting employees in the workplace from biological hazards. Airborne fungi can be detected and identified by an image-acquisition and interpretation system. In this paper we present recent results on the development of an automated image acquisition, probe handling and image-interpretation system for airborne fungi identification. We explain the application domain and describe the development issues. The development strategy and the architecture of the system are described and some results are presented.

Keywords: Microscopic image acquisition, microbiological probe handling, image analysis, image interpretation, case-based object recognition, case-based reasoning.

1 Introduction

Airborne microorganisms are ubiquitously present in various indoor and outdoor environments. The potential implication of fungal contaminants in bio-aerosols on occupational health is recognized as a problem in several working environments. There is a concern on the exposure of workers to bio-aerosols especially in composting facilities, in agriculture, and in municipal waste treatment. The European Commission has therefore guiding rules protecting employees in the workplace from airborne biological hazards. In fact, there are an increasing number of incidents of building-related sickness, especially in offices and residential buildings. Some of these problems are attributed to biological agents, especially in relation to airborne fungal spores. However, the knowledge of health effects of indoor fungal contaminants is still limited. One of the reasons for this limitation is that appropriate methods for rapid and long-time monitoring of airborne microorganisms are not available.

Besides the detection of parameters relevant to occupational and public health, in many controlled environments the number of airborne microorganisms has to be kept below the permissible or recommended values, e.g. in clean rooms, in operating

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theaters, and in domains of the food and pharmaceutical industry. Consequently, the continuous monitoring of airborne biological agents is a necessity for the detection of risks of human health as well as for the flawless operation of technological processes.

At present a variety of methods are used for the detection of fungal spores. The culture-based methods depend on the growth of spores on an agar plate and on the counting of colony-forming units [14]. Culture-independent methods are based on the enumeration of spores under a microscope, the use of a polymerase chain reaction or on DNA hybridization for the detection of fungi [14]. However, all these methods are limited by time-consuming procedures of sample preparation in the laboratory. This paper describes the development and the realization of an automated image-acquisition and probe handling unit of biologically dangerous substances and the automated analysis and interpretation of microscope images of these substances.

In the system described here, contaminated air containing bio-aerosols is collected in a defined volume via a carrier agent. They are recorded by an image-acquisition unit, counted, and classified. Their nature is determined by means of an automated image-analysis and interpretation system. Air samples are automatically acquired, prepared and transferred by a multi-axis servo-system to an image-acquisition unit based on a standard optical microscope with a digital color camera. This part of the system is described in Section 2. To obtain a sufficient image quality, special requirements have to be fulfilled by the image-acquisition unit which will be described in Section 3.

The variability of the biological objects is very broad. Given the constraints of the image acquisition, this variability is found in the appearance of the objects as well. There are no general features allowing one to discern the type of the detected fungi. In the system employed here, images are stored, and a more generalized description for the different appearances of the same objects is used. We will describe this novel case-based reasoning approach for the image analysis and its interpretation in Section 4. Finally, we summarize our work in Section 5.

2 System Requirements

The system to be developed should allow to collect dust and biological aerosols in well-defined volumes over microscope slides, deposit them there, image them with an appropriate method and count and classify them with an automated image analysis and interpretation method, in order to determine the following parameters from the images:

- Total number of airborne particles
- Classification of all particles according to the acquired image features
- Classification of biological particles, e.g. spores, fragments of fungal mycelia, and fragments of insects
- Number of respirable particles
- Total number of airborne particles of biological origin
- Number of dead particles of biological origin
- Number of viable and augmentable particles of biological origin

- Identification of species or geni exploiting the characteristic shapes of spores and pollen
- Proportion of airborne abiotic and biotic particles
- Proportion of dead and viable airborne microorganisms.

At the beginning of the project the following requirements concerning the optical and the mechanical system were defined:

- Color images should be produced in order to facilitate the separation of dead and living objects.
- It should be possible to generate images in at least three defined depths of field.
- A marker liquid like lactophenol should be used to further enhance the separation of dead and living objects (blue color for living objects). For that a cover slip is necessary in order to uniformly distribute the marker drop on the object slide.
- The object slide should be covered with an adhesive in order to fix the airborne germs.

Table 1. Strains of fungi used and selected properties of spores

Species	Strain no.	Spore shape	Spore color	Spore size [µm]
Alternaria alternata	J 37 (A[1])	Septated, clavate to ellipsoidal	Pale brown	18 – 83 × 7-18
Aspergillus niger	i400 (B[2])	Spherical, ornamented with warts and spines	Brown	Ø 3.5 - 5
Rhizopus stolonifer	J 07 (A)	Irregular in shape, often ovoid to elliptical, striate	Pale brown	7-15 × 6-8
Scopulariopsis brevicaulis	J26 (A)	Spherical to ovoid	Rose-brown	5-8 × 5-7
Ulocladium botrytis	i171(B)	Septated, ellipsoidal	Olive-brown	18-38 × 11-20
Wallemia sebi	J 35 (A)	Cubic to globose	Pale-brown	Ø 2.5 – 3.5

[1](A): from culture collection of JenaBios GmbH, Jena, Germany.
[2](B): from the fungal stock collection of the Institute of Microbiology, University of Jena, Jena, Germany.

Six fungal strains representing species with different spore types were identified as important species in different environments (Tab. 1) by our industrial project partner JenaBios GmbH. A database of images from the spores of these species was produced and was the basis of our development. The number of imaged spore per species was about 30-50. Since no commercial system was known fulfilling all requirements, a corresponding system was developed which is described in what follows.

3 The Automated Imaging System

3.1 The Microscopic Image-Acquisition System

Following the specifications given in Section 2 we developed an automated probe-handling and digital image-acquisition system for taking microbiological material from air samples [12]. An existing optical Leitz microscope was upgraded and expanded in its hardware. A lens from Olympus with a magnification of 60X and a numerical aperture of 0.7 was used. Its focal length of 1.7 mm provided sufficient clearance between the lens and the object slide including the cover glass to avoid collisions due to their variability in thickness. The lens was inserted in an autofocusing device from Physik Instrumente (PI, Karlsruhe, Germany) which was mounted on the lens revolver. A motorized xy-table from Märzhäuser (Wetzlar, Germany) with a motion controller was used to arbitrarily shift the object slide in both x and y direction. For the digital image acquisition a 1.4 Mpixel color digital camera from Soft Imaging System (SIS, Münster, Germany) was used. Our estimates showed that a pixel number larger than 1.4 Mpixel is sufficient for the given magnification. Fig. 1 demonstrates that the optical resolution is sufficient to recognize details in spores like Ulocladium.

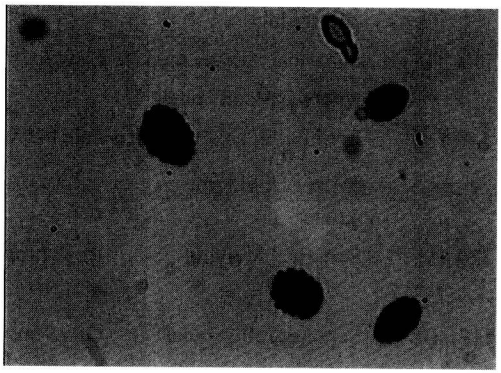

Fig. 1. Image demonstrating the resolution of the optical microscope used. The microscopical image displays spores of Ulocladium. The field of view is 134×100 μm^2. The sample was prepared by AUA/JenaBios, lens Olympus 60X/0.70. The resolution in this image is 5 μm.

The functions of image acquisition and image storage, movement of the specimen in x and y direction, and auto-focusing in z-direction are controlled by the AnalySIS Pro software from SIS. A pattern of images at any image position can be freely programmed and stored in a macro-code. This holds as well for the number of images to be captured. If necessary it is possible to capture automatically images at different depths of focus around the optimum position. By the automatic shading correction, the effect of an inhomogeneous illumination of the object can be removed.

3.2 The Automatic Probe-Acquisition and Handling System

The following chapter describes the main units and functions of the demonstration set-up realized in the course of the project. A stock of special object slides covered with a sticky layer from Umweltanalytik Holbach [1], (Fig. 2) is kept in a slide storage. A sliding gripper takes the lowest slide in the storage and transports it into the slit impactor from Umweltanalytik Holbach (Fig. 3). The object slides are separated by distance holders with a corresponding recess, in order to avoid sticking between the slides. The distance holder is removed by the same gripper, now moving in opposite direction and depositing the distance holder into a box. The distance holders can be used again when the slide deposit is reloaded.

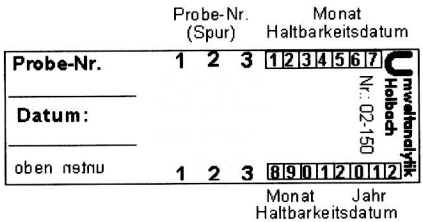

Fig. 2. Object slide of standard size 76×26×1 mm³ with a central sticky layer [1]; Image obtained from Umweltanalytik Holbach

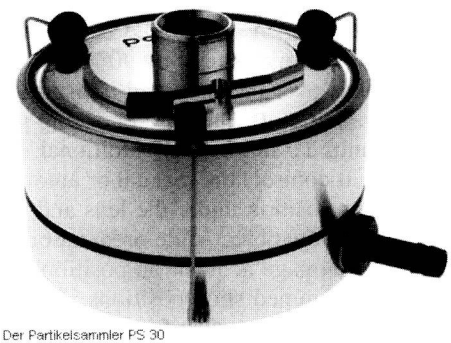

Fig. 3. Slit impactor for collection of airborne particles [1]; Image from Umweltanalytik Holbach

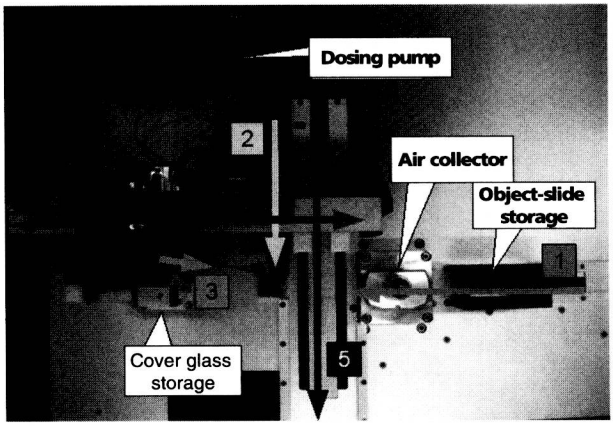

Fig. 4. Top view of the mechanical unit for moving object slides, indicating also the position of the cover-glass storage, the dosing pump for lactophenol, the slit impactor or air collector, and the storage for the object slides. The numbers 1 – 5 indicate the sequences of the movements; axis No. 6 is not shown.

In the slit impactor the air (Fig. 3), potentially containing airborne germs, is guided on the sticky area of the object slide by the air stream generated by a microprocessor controlled air pump. After a few tens of seconds which can be adjusted accordingly, the pump is switched off and the object slide is transported to the pipetting unit driven by the dosing pump (Cavro XL 3000 from Tecan Systems San Jose, Ca, USA. To this aim it has to change its transporting axis and thus its direction of movement. From a thin nozzle one drop of lactophenol is deposited on the sticky area of the object slide which is afterwards transported via the axis crossing to the cover-slip gripper unit. This gripper acts as a low-pressure sucker and takes one cover glass from the deposit and puts it with one edge first on the object slide. Then the cover glass falls down on the object slide and flattens the drop so that it will be distributed all over the sticky area forming a thin layer. In this way the airborne germs collected in the sticky layer are immersed in the lactophenol. In lactophenol living germs get a blue color. The object slide is then transported back to an axis crossing-point where it again changes its direction of movement by 90° and is transported to the xy-table of the microscope which takes over the slide and transports it directly under the lens. The timing of the transportation units, the air and dosing pump is controlled by a distributed multi-axis motion-unit. To this end an additional module was integrated into the AnalySIS Pro software. It controls the manual or automated shift of the xy-table between the image-acquisition position under the lens and the loading position, where the object slide is shifted from the object-slide preparation unit to the xy-table. After the object slide has reached the image acquisition position, the microscope camera then grabs the images at the programmed slide positions after auto-focusing of the microscope lens at each position. The cycle of shifting the xy-table to the defined positions, auto focusing, image acquisition and storage is programmable in a macro-code integrated into the AnalySis Pro software. This can also be done for other procedures like shading correction or image acquisition at different z-positions. After having finished the imaging sequence, the slide is transported away from the xy-table with a special arm

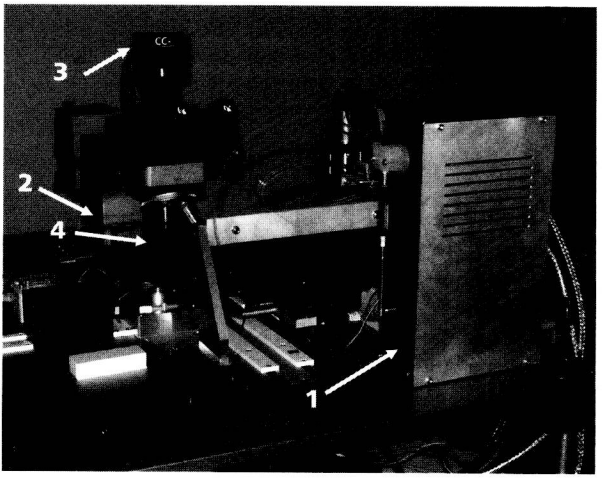

Fig. 5. Prototype set-up showing the dosing pump (arrow 1), several axes, the optical microscope with xy-table (arrow 2), and the digital camera (CC-12, arrow 3). The auto-focusing unit holds the lens (arrow 4).