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**Session 7 - New Methods and Technologies for Medicine and
Biology**

Session 8 - Embedded System Design and Application

Session 9 - Image Processing, Image Analysis and Computer Vision

Session 10 - Mobile Communications

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Preface

Dear Participants,

Confronted with the ever-increasing complexity of technical processes and the growing demands on their efficiency, security and flexibility, the scientific world needs to establish new methods of engineering design and new methods of systems operation. The factors likely to affect the design of the smart systems of the future will doubtless include the following:

- As computational costs decrease, it will be possible to apply more complex algorithms, even in real time. These algorithms will take into account system nonlinearities or provide online optimisation of the system's performance.
- New fields of application will be addressed. Interest is now being expressed, beyond that in "classical" technical systems and processes, in environmental systems or medical and bioengineering applications.
- The boundaries between software and hardware design are being eroded. New design methods will include co-design of software and hardware and even of sensor and actuator components.
- Automation will not only replace human operators but will assist, support and supervise humans so that their work is safe and even more effective.
- Networked systems or swarms will be crucial, requiring improvement of the communication within them and study of how their behaviour can be made globally consistent.
- The issues of security and safety, not only during the operation of systems but also in the course of their design, will continue to increase in importance.

The title "Computer Science meets Automation", borne by the 52nd International Scientific Colloquium (IWK) at the Technische Universität Ilmenau, Germany, expresses the desire of scientists and engineers to rise to these challenges, cooperating closely on innovative methods in the two disciplines of computer science and automation.

The IWK has a long tradition going back as far as 1953. In the years before 1989, a major function of the colloquium was to bring together scientists from both sides of the Iron Curtain. Naturally, bonds were also deepened between the countries from the East. Today, the objective of the colloquium is still to bring researchers together. They come from the eastern and western member states of the European Union, and, indeed, from all over the world. All who wish to share their ideas on the points where "Computer Science meets Automation" are addressed by this colloquium at the Technische Universität Ilmenau.

All the University's Faculties have joined forces to ensure that nothing is left out. Control engineering, information science, cybernetics, communication technology and systems engineering – for all of these and their applications (ranging from biological systems to heavy engineering), the issues are being covered.

Together with all the organizers I should like to thank you for your contributions to the conference, ensuring, as they do, a most interesting colloquium programme of an interdisciplinary nature.

I am looking forward to an inspiring colloquium. It promises to be a fine platform for you to present your research, to address new concepts and to meet colleagues in Ilmenau.



Professor Peter Scharff
Rector, TU Ilmenau



Professor Christoph Ament
Head of Organisation

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Spectral Imaging Technology for Microarray Scanners

INTRODUCTION

In biotechnology fluorescence analysis methods are a growing area of interest. Many techniques have been developed, to investigate biological functions on a molecular level. But conventional methods of microarray analysis are, although suggesting high sensitivity at the detection of genetic material, rather inaccurate, unreliable [1] and slow. Origin of these deficiencies are, e.g., procedural variations at the production of microarrays. Because of the low, unidimensional information content obtained with conventional scanners, the variations can not be identified and assigned to a single source. Here, spectral imaging (SI) technology allows advances through fast, two-dimensional detection of whole fluorescence emission spectra. The benefits of higher information content could be shown in experimental studies for different situations.

SPECTRAL IMAGING PRINCIPLE

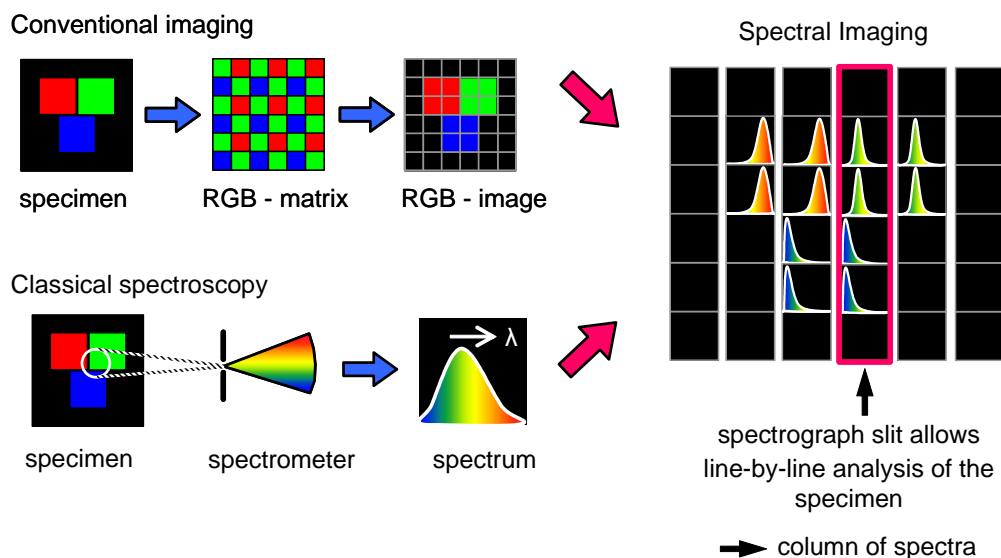


Fig. 1, Spectral imaging as a combination of imaging and spectroscopy

SI is a beneficial combination of optical spectroscopy and digital image processing. The technology originates from airborne remote sensing used by geologists. The emitted ra-

diation to be analyzed goes through an entrance slit and a dispersive element before it falls on the array detector. Here a spatial and a spectral dimension are recorded simultaneously (Fig.1). The third dimension is detected consecutively by scanning the sample. Because the number of spectral channels is in the range from tens to hundreds, our SI-system can be classified as hyperspectral.

MICROARRAY ANALYSIS

The application of SI for microarray scanners allows probing more than one fluorescence label with a single laser excitation source. In gene technology, fluorescently labeled DNA samples are attached to a glass or plastic surface. Through specific binding, DNA probes are hybridized, e.g., to the fixed oligonucleotides. After excitation of the sample the fluorescent emission of different dyes yields biological information about the probes. Gene expression levels provide important information of the state of any organism. To measure it, the hybridized nucleic acids of the microarrays are scanned either point-by-point by a fluorescence microscope or by an imager. One significant drawback of the current technology is channel cross-talk, when the dye number increases [2,3]. Although microarrays are already widely used, solutions must be found to increase the sensitivity, accuracy and throughput. The application of multiple dyes can help with extensive studies. For gene expression experiments with a large number of specimens, including several microarrays, reference standards can be used for indirect comparison [4].

Microarrays of oligonucleotides with four different cyanine dyes Cy3, Cy3.5, Cy5, and Cy5.5 (MWG Biotech) were prepared by the Leibniz Institute for Natural Product Research and Infection Biology (HKI) in Jena and measured by a GenePix 4000B scanner (Molecular Devices) as a reference initially. Investigated, dye labeled oligonucleotides were spotted with dye concentrations ranging from 1 pmol/ μ l to 0.5 fmol/ μ l.

EXPERIMENTAL SETUP

The experimental setup consists of three parts, excitation path, emission path and translation of the sample. The excitation is accomplished by two lasers, a frequency-doubled solid state laser (Soliton DLSOT-100) producing 100 mW of 532 nm radiation and a diode laser (Stocker Yale Lasiris Magnum 501L) with 250 mW at 635 nm in pulsed mode. The SI-system detects the emission within a line-shaped area. To illuminate only that region and to avoid unnecessary degradation of surrounding dye, the two lasers are

equipped with Powell lens line projection optics. This allows an excitation line with a width of less than 40 μm , enhancing the resolution of the SI-system in scan direction.

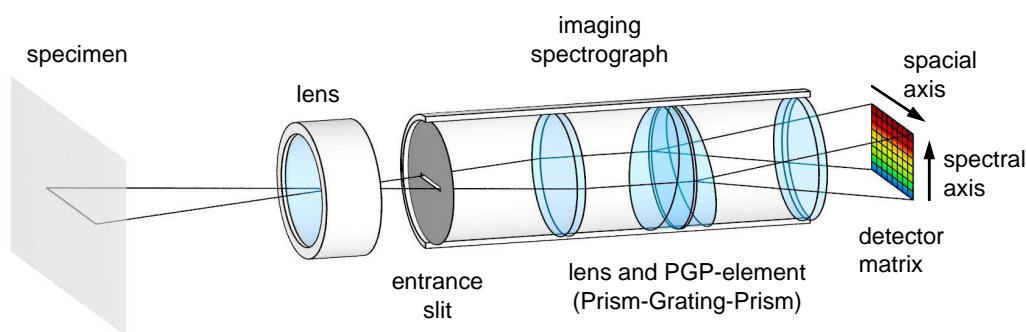


Fig. 2, Schematic diagram of the imaging spectrograph ImSpector (Specim / Finland)

The detection system comprises a lens with emission filter, spectrograph and camera. A macro lens (Schneider-Kreuznach Apo-CPN 4.0/45) is used to realize magnifications from 1:1 to 1:20. Two different emission filters (Semrock LP-01-532RS-25, LP02-647RU-25) suppress scattered light from the lasers. In Fig.2 the principle of the imaging spectrograph (Specim ImSpector V8E) is illustrated. The entrance slit with a length of 14.3 mm was chosen to be 80 μm wide, which is the best compromise between wavelength resolution (6 nm) and adequate light intensity. The fluorescent light is collected by a highly sensitive cooled EMCCD-camera (Andor iXon DV887DCS-BV), capable of single photon detection. Typically, signal intensities of about 12,000 counts are achieved at an excitation energy of 300 μJ . Experiments showed, that the optimum conditions of irradiation power and camera integration time are at 3 mW and 100 ms.

The microarray, having size and shape of a microscope slide, is translated by a motorized translation stage for micrometer steps, perpendicular to the extent of the excitation and detection line. With one measurement, up to 512 spectra are detected simultaneously, having the potential of fast scans. The whole setup can be seen in Fig.3.

COMPONENT ANALYSIS

Component analysis yields a clear discrimination of the fluorescent cyanine dyes, despite strongly overlapping emission spectra. Fluorescence signals of labeled biospots can be seen as linear superimposed. Therefore, data interpretation can be performed by component analysis, which determines the abundance of single spectral signatures [5,6]. The analysis of the whole spectral response of each biospot allows a separation of different dyes with neighbored fluorescence spectra, as is the case with multicolored spots.

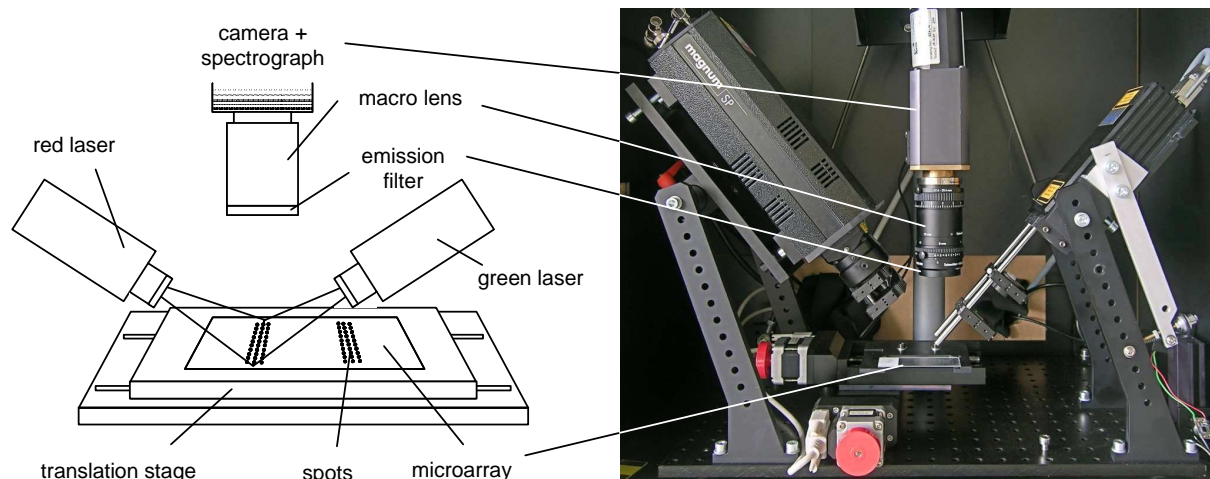


Fig.3, Experimental setup of the spectral imaging microarray scanner

One possible method is linear spectral unmixing, where the emission spectra of all dyes involved have to be known. To avoid the restriction of demanded *a priori* knowledge, a multivariate curve resolution – alternating least squares – algorithm (MCR-ALS) was developed in cooperation with the University of Barcelona [7]. The MCR-ALS only needs sparse *a priori* information, emission spectra are optional. In case they are known, the information can be used as a constraint to accelerate the ALS optimization process. This unmixing procedure does not only calculate quantified fluorescence emissions of the known dyes but also that from initially unknown sources, which can be identified at a later step with their signature. Both unmixing methods yield maps that contain the intensity of all discrete fluorescent emission sources. All calculations were done with Matlab.

EXPERIMENTAL PROCEDURE AND RESULTS

Four-dyes labeled microarrays (HKI) were analyzed by SI. The fluorescent labels were applied in different configurations of both, co-labeling and concentration.

For component analysis of the SI-data, fixed (*a priori*) as well as a number of adjustable signatures were predefined and used with the MCR-ALS algorithm. For excitation at 532 nm three fixed signatures (Cy3/3.5/5) and four adjustable were admitted. At red excitation, three fixed (Cy3.5/5/5.5) and three variable were allowed. In both cases, a non-negativity constraint was assigned.

The analysis yielded clearly distinguishable intensity maps illustrated in Fig.4. The fundamental emission spectra (a) and (d) found on the biochip were used as signature libraries with the MCR-ALS unmixing procedure. Result of this calculation were intensity maps (b) and (e) for every signature. Two or three of these maps can be combined to a RG(B)-image (c) and (f) that easily shows the co-occurrence of concentrated dye.

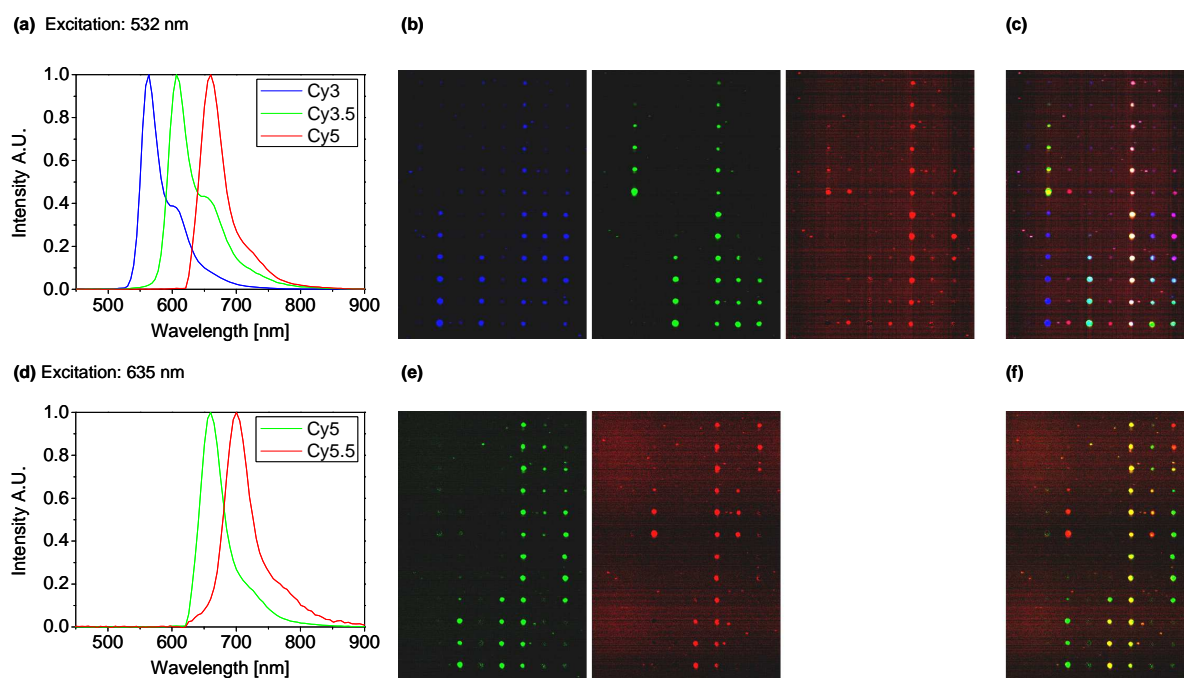


Fig. 4, Fluorescence spectra (a,d), raw intensity maps of Cy3, Cy3.5, Cy5 and Cy5.5 (b,e) and combined intensity maps (c,f), which show the co-occurrence of dye

We also investigated conventional twofold labeled spots. False-positive signals, based on channel crosstalk, were determined by the analysis of 616 spots from a microarray scanner calibration slide (Full Moon BioSystems DS01), 308 of which consisted of Cy3 and the other half of Cy5. The spots were concentrated from $1.1 \cdot 10^{-3}$ to $1.5 \cdot 10^5$ fluorophores/ μm^2 . The slide was analyzed with GenePix and SI-system. After component analysis by MCR-ALS (non-negativity and equality constraint, one variable and two pre-assigned signatures), the grey value images of both measuring methods were analyzed for average spot intensity. To obtain the relative false-positive error, the average intensity of all equally concentrated Cy5-spots was divided by the associated Cy3-spots after subtraction of the spots interspace signal (background).

The analysis of false-positive signals at 532 nm excitation yielded a relative error of $6 \cdot 10^{-6}$ (SI) and $1.5 \cdot 10^{-3}$ (GenePix) at high dye concentrations. At very low amounts of fluorescent label the error was above 10% for GenePix and 5% for SI. On average, the error of the SI-System was found to be smaller by a factor of 3.5 (Fig.5). Comparable results were found at the analysis of twofold labeled microarrays made by the HKI. Here the average false-positive error of 2,7% (GenePix) could be reduced to 0,8% (SI).

Besides the samples described, we have made successful investigations on alternative fluorescent dyes Rhodamine 6G and Tetramethylrhodamine. Furthermore, we analyzed high density (37,632 spots) microarrays from the Netherlands Cancer Institute (NKI).

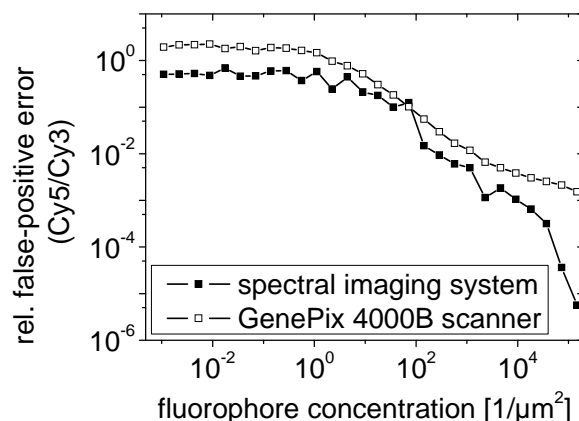


Fig. 5, Comparison of relative false-positive signals of different concentrated Cy5-spots

CONCLUSION

The spectral resolved measurement of fluorescence emission of biospots by SI-technology shows clear advantages in comparison to conventional filter-based microarray scanners. We could show an efficient detection and discrimination of the fluorescent dyes Cy3, Cy3.5 and Cy5 by green laser excitation and Cy5 and Cy5.5 by red excitation, which is impossible with a conventional filter-based microarray scanner. We also found a reduction of false-positive results for twofold labeled microarrays. Summarized, our SI-technology is able to differentiate clearly between three overlapping spectral dye-signatures besides disturbances. SI has the potential to enhance the accuracy, reliability, and speed of microarray scanners, because the fluorescence of many biospots can be measured simultaneously as well as spectral disturbances of the background.

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