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Chip Technology in Analytical Chemistry*

The promises of chip technology in analytical chemistry are attractive: nanoliter-size sample volumes, low reagent consumption, high degree of multiplexing, short analysis times, ultra-low detection limits, ease in portability of process and result. It is predictable that chip technology will result in a significant cut of costs per information unit obtained whilst at the same time will improve the quality and validity of data obtained.
A new era of massive parallel information generation will pave the way to novel, much more efficient approaches in research and development. Chip-based technologies will expedite the identification and development of drug candidates and be the tools for more precise identification, monitoring, and treatment of both gene-based and infectious diseases. This is made possible, on one hand, by the massively parallel detection of a multitude of biological markers and genetic predisposition parameters so far inaccessible to analysis systems and, on the other hand, by making decisions based on a much broader basis of pharmacologically and clinically significant parameters.
Research approaches for chip-based analytical systems started about ten years ago and are focused on two main streams: chips for fluidic handling and separations and chips for detection of analytes. On the detection side, products are already on the market, *e.g.* genechips enabling information to be obtained on the presence of genetic variations and genetic defects based on the binding of DNA to over 50 000 different oligonucleotide probes immobilized on one chip. On the fluidic and separation side several companies have developed systems that are close to commercialization.
The workshop gave a broad overview covering the current technical status of chip-based analytical systems, key applications in clinical research, in drug metabolism, and in the field of infectious diseases and included a critical discussion about current limitations as well as the future potential and value of bioinformatics. The outline of chip-based combinatorial chemistry approaches and novel high density enzymatic assays showed that 'classical' chip technology is already expanding to new fields of applications. Abstracts by the authors are given below.

Keywords: Analytical chemistry · Chip technology · ILMAC · Microarrays

Application of Chip Technology in Preclinical and Clinical Research

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Recent advances in the field of genomics and genetics open up new horizons for medicine. Interindividual variation of drug response (pharmacogenetics) or toxic reactions to chemicals (toxicogenetics) are frequently due to genetic polymorphisms of drug-metabolizing enzymes, receptors or transporters. The use of this genetic information in preclinical and clinical drug development and patient management was discussed with examples of therapeutic problems with commonly used drugs.
Other areas of clinical research strongly influenced by recent advances in genomics are the challenges of understanding multigenic or multifactorial diseases, the study of the effects of drugs on gene expression and the testing of microbial genomes (*e.g.* HIV) for resistance to therapy.

The complete understanding of individual differences in drug response and xenobiotic toxicity or therapeutic efficacy and of genetic susceptibility to develop certain diseases requires highly reliable and efficient methods to assess genetic variation.
Microarray technologies or DNA probe arrays have been used to rapidly resequence known genes and discover genetic variants, to detect the presence of mutations or single nucleotide polymorphisms (SNPs) or to analyze the expressions of hundreds or thousands of individual genes.
At the present time, microarrays are used mainly as research tools to test for individual genetic profiles. It is predicted that they will become routine analytical tools in the prophylaxis, diagnosis, and treatment of disease.

Microarray Applications in Infectious Disease Research

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An oligonucleotide array of more than 250 000 specific probes representing two complete bacterial genomes including the 1743 open reading frames of *Haemophilus influenzae* and the 1969 open reading frames of *Streptococcus pneumoniae* has been designed. However, the eukaryotic transcript labeling methods

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developed by Affymetrix are not directly applicable to prokaryotes due to the absence of polyadenylation in bacterial messenger RNA. We optimized a random reverse transcription method that allows detection of transcript abundance for all genes and was shown to be sensitive, specific, quantitative, and reproducible. Genome-wide transcriptional analysis allows differential expression of all genes of a bacterium to be measured upon external stimulation. The chip is therefore the ideal tool to analyze how pathogens react in response to antibacterial compounds. The data generated is used to predict the mode(s) of action of compounds by comparison of the transcriptional profile they induce in cells with a database containing the transcriptional profiles of bacteria treated with antibiotics of known modes of action. It is also used to gain a better understanding of the underlying mechanistic differences between compounds that trigger bacterial lysis (bacteriocidal), and those that simply arrest growth (bacteriostatic). Currently the cellular processes leading from site of drug action to cell lysis are a black box. Moreover, we have also shown how an oligonucleotide chip can be used as a sequence screening tool, and allelic variation screening tool.

Gene Expression Data: An Informatics Perspective

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The major focus of toxicogenetics is the study of differential gene expression induced as an adaptation or 'change-of-plans' response to chemical or environmental stress. The fundamental adaptation of toxicogenetics is that there are no toxicologically relevant outcomes *in vitro* or *in vivo*, with the possible exception of rapid necrosis, which do not require differential gene expression. Additionally, most toxicologically relevant outcomes require differential expression of multiple genes. By studying patterns of gene expression we can learn a great deal about the fundamental mechanisms of chemical toxicity. However, the measurement, management, and analysis of gene expression data requires sophisticated informatics systems, and there are interesting and important challenges related to each of these informatic tasks.
The presentation, delivered from the perspective of an information scientist, identified and described challenges involved in measuring, managing, and analyzing gene expression data. The presentation was based on the lessons PHASE-1 has learned from years of work capturing, storing, and analyzing gene expression data for toxicology. Examples of issues we have found include: Is 'fold induction' a useful measurement? What is the best way to normalize gene expression levels in an experiment? What does 'similarity' mean with respect to gene expression patterns? Is gene expression comparable from one platform technology (such as glass slides) to another (such as membranes)?

Microchemistry and Analysis on Chips

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The large number of new biological targets originating from genomics research has driven the demands for new compounds. Combinatorial chemistry has increased compound production, but greater quality of products *via* analytical verification of structure has become expected. Coupled with analytical verification, throughput capacities and decreased reagent usage have driven miniaturization, higher density plate formats, and enhanced automation. Similar concerns have also impacted other preclinical drug discovery programs including high-throughput screening, ADME, and toxicology. Orchid Biocomputer has developed a platform technology for the seamless integration and miniaturization of preclinical drug discovery research. Key components of this platform are a ChemStream™ synthesis chip and a MassStream™ analytical chip. The ChemStream will accommodate a variety of Chemtel™ chips (96, 34, 1536) which can be used independently or modularly combined to generate up to 12 000 discrete compounds.
The Chemtel chips incorporate microfabricated components for valving and pumping of fluids, integrated within a three-dimensional fluidic network. The pumping and valving mechanisms have no moving parts, making large-scale integration feasible and inherently reliable. Additional features such as temperature control have expanded the utility of these chips in chemical synthesis. The MassStream 96 chip will provide purity assessment and molecular weight confirmation of products using less than 0.1 nmol and cycle times as low as 15 seconds. The macro to micro interface to the chip is a key area of research and conventional tube and valve design *versus* integration of conventional robotic liquid handlers was discussed.

Industrial Design of Enzymatic Assays in High-Density Formats

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A series of applications in both genetic analysis and genomics needs to be done in a highly parallel format in order to achieve the required throughput. High-density arrays, in principle, seem to be one of the most useful approaches to these tasks. In this presentation, assay formats were discussed that are based on an enzymatic reaction in homogeneous phase in addition to a specific hybridization. This approach offers a much greater selectivity through the high specificity of the enzymatic reaction. As a first example, a 5'-exonuclease assay was described for use in geno-typing. The scale of the assay has been reduced from a typical microliter range to nanoliter wells. Fluorescence resonant energy transfer was used for detection. The implications for the industrial design of this assay was discussed.
The other examples that were presented deal with ligase-based assays for the detection of single nucleotide polymorphisms (SNP). These assays include two steps. The first step in homogeneous solution includes a hybridization and a ligation reaction of two primers to the target sequence. The first, so-called discriminating primer varies in the terminal base. Ligation can only be expected when the terminal base matches with the target sequence. The other, so-called common primer is fluorescently labeled and, hence, the ligation product is as well. In addition, the discriminating primer is coded with a short artificial oligo sequence which is complementary to chip-bound capture oligos. Thus, the ligase products can, in a second step, be hybridized to a chip. By reading the fluorescence signals, positive base-pair matches can be detected. The same principle can be applied to detect both alleles in one assay using two different fluorescence labels. The whole concept, which enables a universal chip to be manufactured, was discussed in terms of its industrial design features.