

MICROARRAYS FOR DETECTION OF LOW ABUNDANCE GENES & PROTEINSM. Ehrat, A. Abel M. Pawlak*Zeptosens AG, Witterswil, Switzerland*

INTRODUCTION: The breakthroughs in genomics in the last years – in many cases based on the massively parallel generation and analysis of biological information - has been made possible to a large extent by the development of miniaturized and multiplexed analysis systems, among them DNA microarrays. Today, protein chips will be one of the key approaches to bridge the gap between genomics and proteomics by delivering functional information on gene expression. The development and broad dissemination of new technologies therefore will be crucial to further advance in genomics based disciplines of life sciences. Moreover, analysis of genes and their products for drug discovery and development, as well as for disease diagnostics place new demands on the sensitivity, speed and reliability of new analytical systems.

Zeptosens' goal to significantly contribute to that endeavour by initiating a new era in highly multiplexed, automated and ultrasensitive bimolecular analysis on nanotechnology-based microarrays and readout systems. With the ZeptoTM-product line, Zeptosens is developing and will introduce a new revolutionary fluorescence-based detection technology for nucleic acid and protein microarrays setting new standards in microarray readout performance in terms of detection limits, quantification and automation.

Technology: Zeptosens is developing a planar waveguide (PWG) technology based microarray readout system. In planar waveguide technology only surface-confined fluorescence labels are selectively excited for emission, bulk medium is not excited. Laser light is coupled via a diffractive grating into a thin film of high-refractive-index material deposited on a transparent support. A strong evanescent field is created, which has only a limited penetration depth of about 200 nm into the adjacent medium. This spacial discrimination results in a significant increase in signal/noise ratios compared with conventional optical detection methods and permits highly selective and sensitive measurements even in the presence of free fluorophores or scattering matrix (e.g. serum).

DNA-Arrays: Mid-density microarrays utilizing oligonucleotides or cDNAs as recognition elements have significant advantages if new and unknown target sequences are to be investigated, if high flexibility is required, or if only the expression level of a few hundred of target genes is to be queried for "focused" target studies. Up to now, however, only a minute amount of the information that could be derived out of the expression level of mRNA has been harvested: Typically, cells contain about 10 pg of total RNA, out of which about 100–500 fg are being represented by mRNA. However, 95 % of this mRNA material are composed out of low abundant genes with less than 10 copies per cell which can be monitored only with high

effort in target and signal amplification. ZeptoGENETM chips permit highly sensitive genomic analysis with no or only linear target amplification. Low abundant genes, expressed in as little as 100 cells, could be monitored. This allows sampling via needle biopsies, laser dissection, and cultivation in miniaturized systems.

Protein Microarrays: The efficient multi-parameter analysis of high sample numbers – healthy versus disease ones or treated versus untreated ones - remains the key to finding and monitoring pharmaceutically relevant target or marker proteins. Zeptosens microarray technology provide the ability to detect low abundant protein markers in a quantitative manner. The microarray platform was designed to provide a comprehensive solution. The concept includes the highest sensitivity, multi-colour fluorescence reader, high quality microarray chips with integrated flow-through micro-fluidics (15 µl chamber volume) adapted to the micro-titer plate format for automated high-throughput measurements. This platform is the base for high performance multiplexed assays with improved biological information content. The sensitivity of the system, enabled by surface-confined fluorescence detection on planar waveguides, is designed to cover the requirements of a wide range of multiplexed microarray applications, without being compromised by instrumental parameters. The detection of down to 1 zeptomole of labelled protein (600 proteins, 3000 labels) on a single spot was demonstrated. The dynamic range of the system, determined to be a factor of 10⁵ through variation of the capture probe density in a spot (10³ proteins at the detection limit up to 10⁸ molecules at full monolayer coverage) times the signal dynamics of 10³-10⁴ within an image, satisfies the needs for the analysis of low to high abundance protein expression levels or ligand-receptor affinity interactions from µM to fM. The waveguide-based read-out principle allows simple "mix & measure" protein assay formats. Measurement in full serum or blood is well possible, as demonstrated for multiplexed cytokine marker analysis. Assay sensitivities of down to 1-10 pg/ml of analyte in serum have been demonstrated. The ability of in situ measurement without additional washing steps is a special benefit for simple assay protocols. ZeptoMARKTM protein microarrays include medium density - medium throughput antibody arrays (1000 spots per array) for target and marker finding in pharmaceutical R&D, and low density, predefined or customized microarray chips (10-50 analytes, high throughput with a multitude of identical arrays per carrier) for marker validation and monitoring in (pre-) clinical drug development.