Protein Arrays
High Throughput Protein Quantification from Total Cell Lysates

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Zeptosens at a Glance

- 1999 Zeptosens AG operational, PWG licensed from Novartis
- 2001 Established production facility for microarrays and readers
- 2002 SensiChip DNA product line launched
- 2003 ZeptoMARK Protein product launched
- 2004 30th patent application on bioarrays filed
- 2005 Bayer Technology Services GmbH acquires assets and business of Zeptosens AG
Our Expertise:
Bioarray Design, Development, Production and Service

- Infrastructure
  - Clean room and assay development labs
  - DNA and protein microarray production and measurement equipment
  - Biomarker profiling study, data analysis and reporting processes
Our Expertise: Bioarray Design, Development, Production and Service

- **Know-how**
  - Surface based protein, peptide and DNA assays
  - Established design, manufacturing and quality control processes for microarrays
  - Microarrays image and data analysis

- **Proven Record**
  - Developed > 50 protein capture micro array assays
  - Developed > 160 assays for reverse arrays in pharmaceutical analysis
  - Performed > 15 protein profiling studies for pharmaceutical companies
  - Applied > 30 patents on PWG and assay technology
Our Expertise: Optical Detection Technologies

- **Infrastructure**
  - Optics development labs
  - Final assembly and QC line for PWG readers with CE and ETL certification
  - Process documentation system according to ISO 9001 standards

- **Know-how / network**
  - Network to international experts and institutes in optics and microoptics for state of the art development of instrumentation
  - Design and realization of advanced optical concepts
  - CE/ETL certification processes

- **Proven Record**
  - Commercial PWG system developed and launched starting from paper concept of technology
Zeptosens’ Product Lines

SensiChip® DNA Microarrays
ZeptoMARK™ Protein Microarrays

ZeptoREADER™

ZeptoMARK protein profiling service

ZeptoVIEW™ image analysis

Assay reagents & protocols
Planar Waveguide Principle - for High Sensitivity Fluorescence Microarray Detection
Fluorescence Excitation in the Evanescent Field for Highest Detection Sensitivity

conventional excitation

ZeptoREADER™ - evanescent detection

- High sensitivity - more information
- Fast time to result
- Less sample preparation
- Direct measurement in blood or serum
ZeptoREADER

Main features of the reader:

• Fully automated unattended chip readout of up to 360 microarrays
• Sophisticated control software
• Excitation lasers: 635nm, 532nm,
• Highest Sensitivity due to planar waveguide technology (1 zmol)
• Typical processing time for 30 microarrays: 30-45 minutes
• Integrated barcode reader for data traceability
• One output file per chip containing all image data, chip information and measurement parameters; compatible with image analysis software
ZeptoCarrier

- Integrated microfluidics
- Automation capability
- Easy handling
- Capacity: 6 ZeptoCHIPS / 36 arrays
Protein Microarrays – Two Formats

Capture Arrays

- Array of target-specific capture molecules (e.g. antibodies)
- Cell extracts contacted with array
- Specific detection via labeled secondary Ab (ELISA-type)

Two Ab’s per target

Cell Lysate Arrays

- Array of samples
- Lysate samples spotted
- Target proteins on chip
- Specific detection with target-specific antibodies

One Ab per target
Capture Arrays
Parallel Fluorescence Immunoassay

- Pre-incubation mix
- Propagating excitation light
- Evanescent field
- Buffer phase
- Waveguide layer
- Glass substrate
- Light intensity
- Distance from surface

- h-IL2
- h-IL4
- h-IL6

- Solution
Quantitative Parallel Assay Needs

- Selected high quality content:
  - High affinity Ab pairs
  - Highly specific Abs
  - No cross-reactivities

- Robust parallel assay on the platform

- Matrix compatibility to achieve good accuracy and precision

- Sensitive readout
  - to reach clinical relevant working range of (low) marker conc.
  - to overcome sample size limitations

- Assay Validation
## Performance Cytokine Antibody Array

<table>
<thead>
<tr>
<th>Name (all human)</th>
<th>Abbr.</th>
<th>LOD* (pg/ml)</th>
<th>LOQ** (pg/ml)</th>
<th>Dose CV*** (%)</th>
<th>Working range (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte colony stimulating factor</td>
<td>G-CSF</td>
<td>1.7</td>
<td>4.6</td>
<td>11</td>
<td>2 - 16000</td>
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<tr>
<td>Interferon- gamma</td>
<td>IFNγ</td>
<td>1.5</td>
<td>5.4</td>
<td>18</td>
<td>2 - 15000</td>
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<tr>
<td>Interleukin-1 beta</td>
<td>IL-1β</td>
<td>0.3</td>
<td>0.6</td>
<td>10</td>
<td>0.3 - 12500</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>IL-2</td>
<td>1.5</td>
<td>2.4</td>
<td>21</td>
<td>1.5 - 22000</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>IL-4</td>
<td>4.3</td>
<td>6.7</td>
<td>13</td>
<td>5 - 19000</td>
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<tr>
<td>Interleukin-6</td>
<td>IL-6</td>
<td>6.0</td>
<td>17</td>
<td>14</td>
<td>6 - 25000</td>
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<tr>
<td>Interleukin-7</td>
<td>IL-7</td>
<td>6.7</td>
<td>12</td>
<td>15</td>
<td>7 - 7200</td>
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<tr>
<td>Interleukin-8</td>
<td>IL-8</td>
<td>3.5</td>
<td>4.1</td>
<td>11</td>
<td>4 - 21000</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>IL-10</td>
<td>0.6</td>
<td>1.4</td>
<td>23</td>
<td>0.6 - 15000</td>
</tr>
<tr>
<td>Tumor necrosis factor-alpha</td>
<td>TNFα</td>
<td>0.7</td>
<td>1.6</td>
<td>8</td>
<td>0.7 - 10000</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>VEGF</td>
<td>6.6</td>
<td>14.2</td>
<td>12</td>
<td>7 - 22000</td>
</tr>
</tbody>
</table>

* Analyte concentration at assay signal of blank plus 2x standard deviation of blank
** Analyte concentration at assay signal of blank plus 8x standard deviation of blank
*** Coefficient of variation of dose, determined from standard deviation of calibration curve signal (standard deviation of replicate spot signals) for concentrations > LOQ

- Shelf life: Comparable assay performance data (LOD/LOQ, Dose CV) after 4 months chip storage at 4°C shown.
Breast Cancer Marker Chip
Validation of a Parallel Cancer Marker Array

50 human breast cancer cytosols – ELISA as a reference

<table>
<thead>
<tr>
<th></th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZeptoMARK</td>
<td>ELISA</td>
</tr>
<tr>
<td>uPA</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.045</td>
<td>0.22</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.001</td>
<td>0.006</td>
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</tbody>
</table>

In collaboration with Oncoscore
Reverse Arrays
Zeptosens Cell Lysate Arrays – From Cells to Protein Profiles

- **Sample**
  - ~10^6 cells
  - ~1 mg tissue

- **Lysis**
  - 50 µl lysis volume

- **Spotting**
  - 400 pL spotting volume
Optimized Chip and Array Layout

6 Arrays / Chip

Ref (●) = reference spots containing constant fluorescence up to 32 samples, in 4 dilutions, in duplicates, or 128 samples, in 1 dilution (not shown)
Array Layout

32 samples spotted in duplicates and at 4 concentrations; 4x22 reference spots

e.g sample #1

Column with reference spots

Line profile (as depicted in image)

controls

Fluorescence

Distance (pixel)
Zeptosens Cell Lysate Arrays – From Cells to Protein Profiles

Cellular systems → Drug treatment → Cell lysis → Spotting → Image Analysis → Readout → Assay → Blocking
Multiplexed Monitoring of Signaling Events

Treatment

Effect

From Cell Signaling Technology
Assay and Readout

Microarray & Image Analysis
ZeptoREADER™
ZeptoVIEW™

Protein expression / activation profiles
ZeptoMARK® CeLyA – Excellent Correlation with Today’s Gold Standard

Good correlation between Western Blot and CeLyA

In collaboration with Hoffmann-La Roche
CeLyA - Testing Efficacy of Drug Candidates

Typical studies comprise: 30 to 60 samples, 30 to 80 proteins corresponding 14,400 to 76,800 data points

Customer study: Impact of 6 drugs and drug candidates on

- 20 different signal pathway proteins in
- 9 different cell lines with
- 3 different stimulants
- 5 drugs: 1 concentration
- 1 drug: 2 different concentrations

In collaboration with Novartis Pharma AG
β-Actin Assay for Array Quality Control (Green)

- β-actin level is comparable for all treatments within a cell line
- All samples were reproducibly spotted
Study Results – e.g. phospho-Erk2

In collaboration with Novartis Pharma AG

Highly sensitive multi-parallel protein expression / activation profiling

From Cell Signaling Technology
Time-Course Information on Pathway Activation

Kinetic profiling (0-60 min) of MAP Kinase activation

- Jurkat cell lysates upon stimulation with $\alpha$CD3/$\alpha$CD28.
- Levels of phosphorylated and total p44/42 Erk examined using corresponding specific antibodies

Array precision allows the quantification of 10-20% changes of phosphorylation
Bar Plot Profile for Protein X

- Cell Line A
  - Compound 1: Decreasing concentration
  - Compound 2: Decreasing concentration

- Cell Line B
  - Compound 3: Decreasing concentration
  - Compound 4: Decreasing concentration

RFI

- Cell Line 1
  - Compound 1: decreasing concentration
  - Control: decreasing concentration

- Cell Line 2
  - Compound 3: decreasing concentration
  - Control: decreasing concentration
Tissues – Profiling of Human Colorectal Cancers

Goal: Identification of markers towards targeted therapy

- Colorectal cancer samples under well controlled conditions (control, treated)
- Lysates produced from fresh patient tissues
- First expression profilings done for 14 (phospho-) proteins

In collaboration with Inselspital, Switzerland

Antibodies from Cell Signaling Technology®
Tissues – Profiling of Human Colorectal Cancers

Goal: Identification of markers towards targeted therapy

First hits of potential markers detected
Verification on a larger set of patients ongoing

In collaboration with Inselspital, Switzerland
ZeptoMARK® CeLyA – Economic Efficiency

Zeptosens CeLyA vs. Western Blot
32 Samples, 4 Dilutions and 100 Target Proteins

<table>
<thead>
<tr>
<th>Category</th>
<th>CeLyA</th>
<th>WB</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per Data Point</td>
<td>0.80</td>
<td>16.00</td>
<td>95% lower cost</td>
</tr>
<tr>
<td>Total Sample Protein</td>
<td>90 µg</td>
<td>1000 µg</td>
<td>90% less sample</td>
</tr>
<tr>
<td>Ab consumption</td>
<td>0.02 ml</td>
<td>0.5 ml</td>
<td>95% less reagent</td>
</tr>
<tr>
<td>Time to result</td>
<td>10 days</td>
<td>100 days</td>
<td>10 times faster</td>
</tr>
</tbody>
</table>