

Planar Waveguides: How Nano Layers Enable to Detect Zepto Moles of Macro Molecules in Pico Liter Spots on Micro Arrays

Dr. Markus Ehrat
Zeptosens – A Division of Bayer Schweiz AG

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Engelberg

What are Biochemical Microarrays?



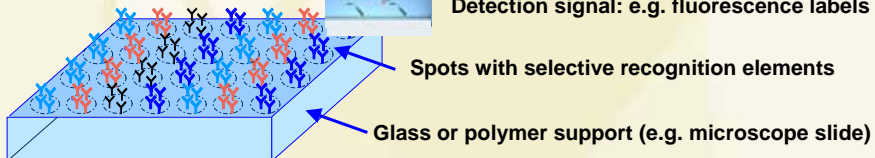
10'000 to 100'000 spots per cm^2 = "photo lithography" arrays



100 to 10'000 spots per cm^2 = spotted arrays

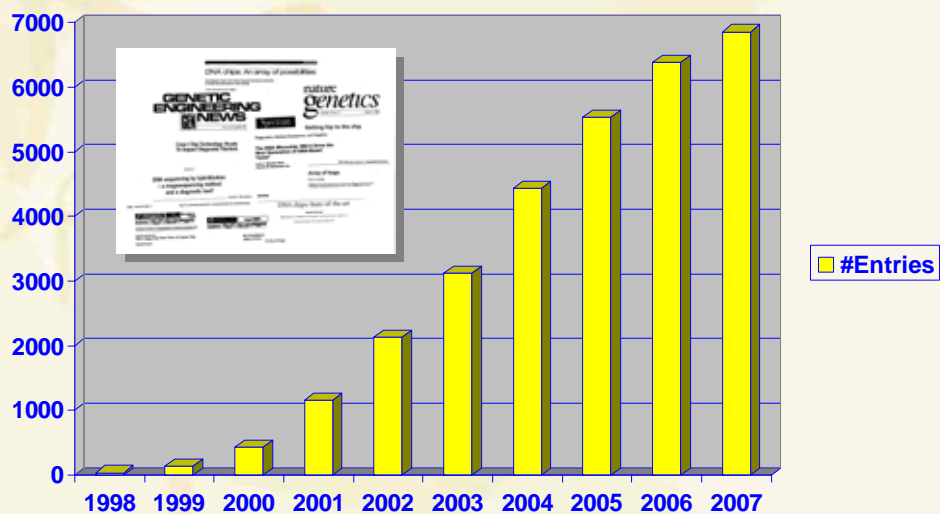


Each spot binds a specific analyte of
the sample solution
Detection signal: e.g. fluorescence labels



**Microarrays -
A powerful technology to measure
thousands of samples and
thousands of analytes
- genes or proteins -,
in a short period of time**

Entries in PubMed Database, Search Term „Microarray“



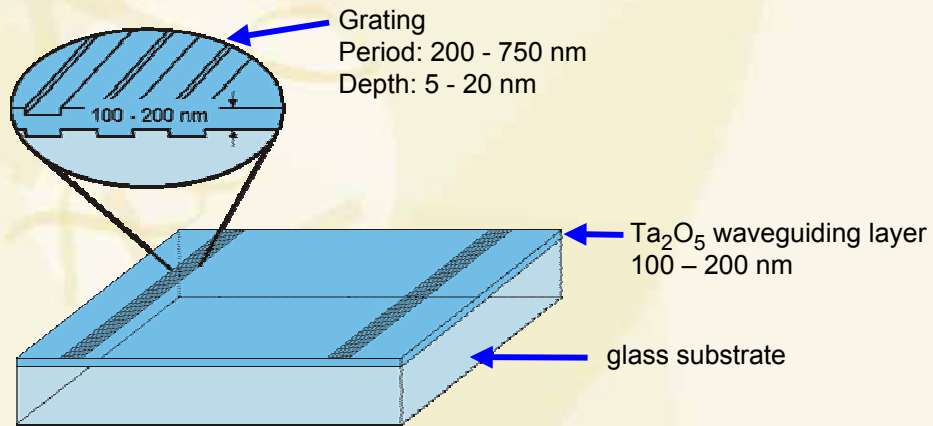
Information Obtained from 200 μ L of Sample

	Detection area (mm ²)	Information per cm ² *
96 well plate	40	1
1536 well plate	2.5	16
150 μ m spot microarray	1.7×10^{-2}	4500
15 μ m spot microarray	1.7×10^{-4}	450'000
1.5 μ m spot microarray	1.7×10^{-6}	45'000'000

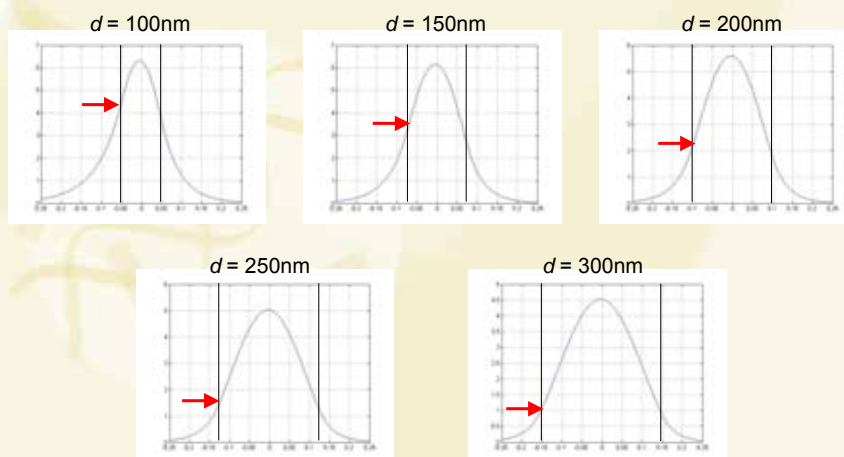
* Sample solution of 200 μ L

**Microarrays -
Small detection areas
Nanoliters of sample volumes:
Require high detection sensitivity**

Thin Film Planar Waveguide (PWG) Chip Design



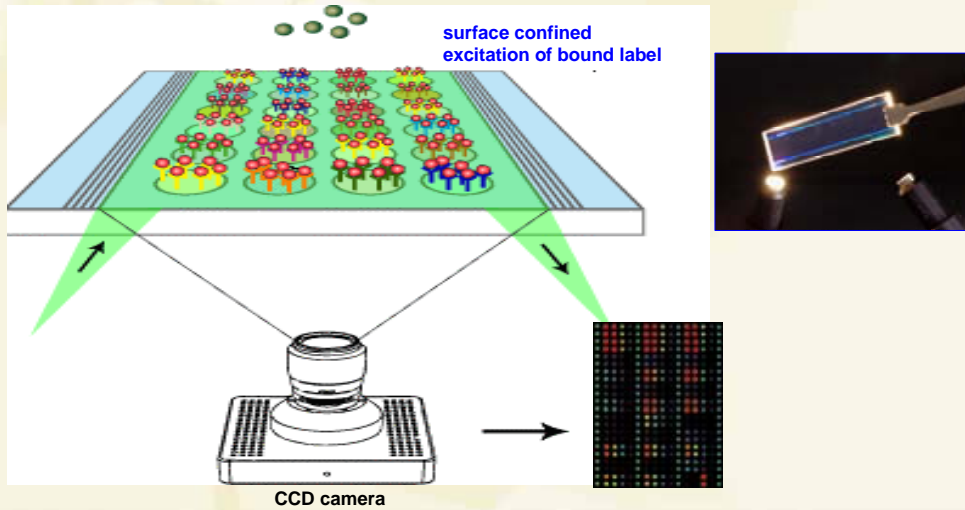
Light Intensity vs. Waveguide Thickness



Parameters:

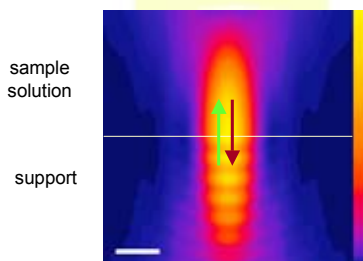
$$n_{\text{sub}}=1.52, n_{\text{sup}}=1.335, n_{\text{PWG}}=2.15, m=0, \lambda=635\text{nm}$$

Planar Waveguide Principle - High Sensitivity Fluorescence Microarray Detection



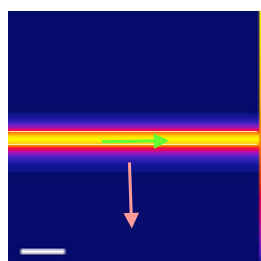
Advantages of Fluorescence Excitation on PWG

Conventional excitation



Confocal excitation:
 Focus depth ~ 2 μ m

ZeptoREADER™ - Evanescent excitation



Evanescent excitation:
 Depth ~ 100nm

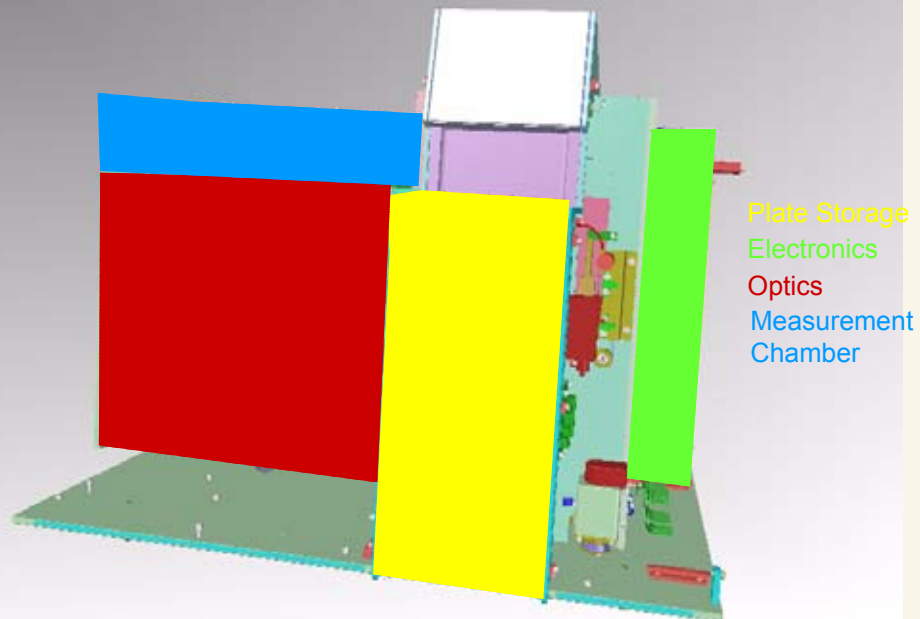
- Separation of excitation and detection directions
- Ultimate sensitivity
- Fast time to result
- Less sample preparation
- Direct measurement in blood or serum

ZeptoREADER – PWG Inside

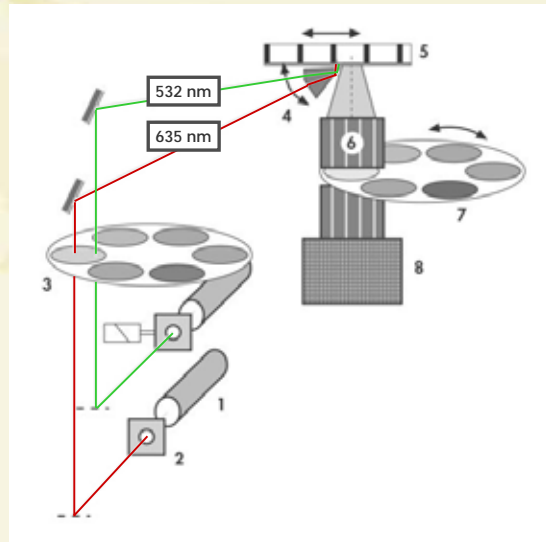


- **Ultra sensitive:**
Planar wave guide technology based evanescent field fluorescence excitation
- **Exceptionally fast:**
Over 120'000 data points in 6 hours
- **Increase efficiency:**
Extended walk away time using 60 slides integrated autoloader
- **Absolutely reliable:**
Swiss designed and manufactured for highest quality and precision possible

ZeptoREADER Setup



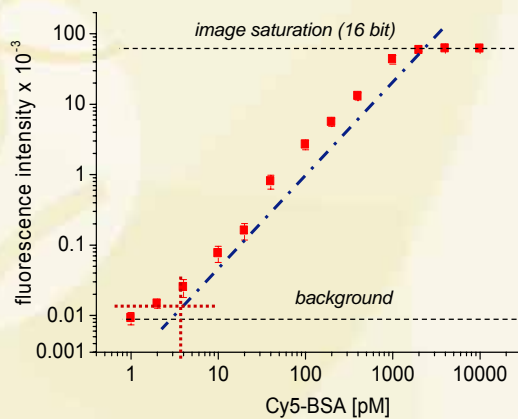
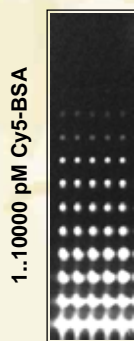
Optical Scheme of the ZeptoREADER™



1. Laser
2. Shutter
3. Gray filter wheel
4. Coupling unit
5. ZeptoCHIP™
6. Front lens unit
7. Emission filter wheel
8. Camera lens unit and CCD

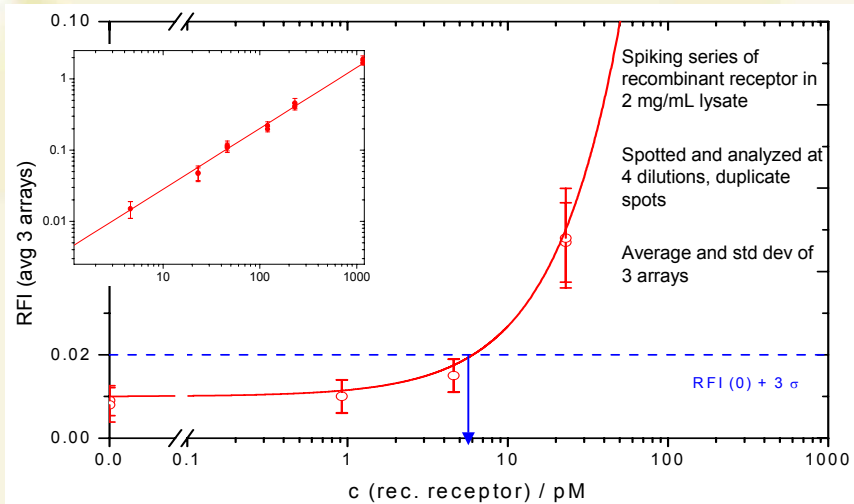
High Sensitivity of PWG Signal Detection

Dilution series



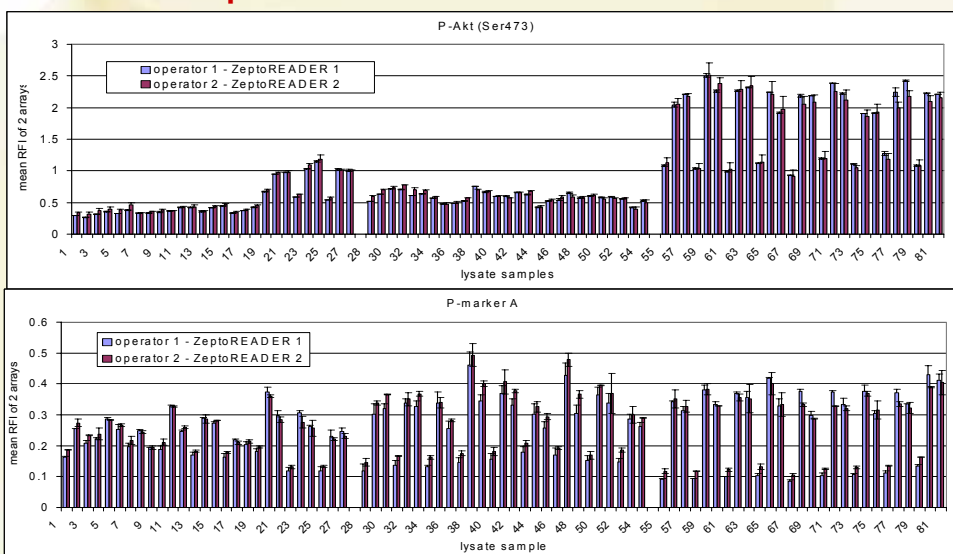
➔ **LOD = 1 zeptomol (600 proteins) per spot**

ZeptoMARK Assay Sensitivity



➔ LOD of ~2000 receptor protein molecules per spot

Assay Reproducibility: Different Operators – Different Days – Different ZeptoREADERS



High detection sensitivity

What is the value in the real world?

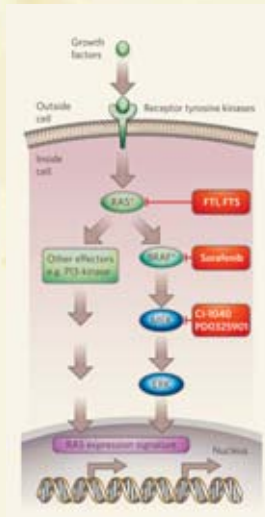
The Systems Approach

- "...the **rate of mortality** from cancer has changed very little over the past 50 years"
- "Cancer therapies are still essentially "**one size fits all**",.....".
- "**Targeting systems**, rather than single molecules, will likely result in **more durable responses** in cancers considered non-responsive to treatment."
- "Thus the "omic" technology is promising an approach both
 - to evaluate the **heterogeneity of cancer patients**
 - and as a means of **identifying biosystems as target for new drug development**"



Science, Vol 312, May 26, pp 1157, 1165, 1166, (2006)

Signatures Guide Drug Choice **Julian Downward**

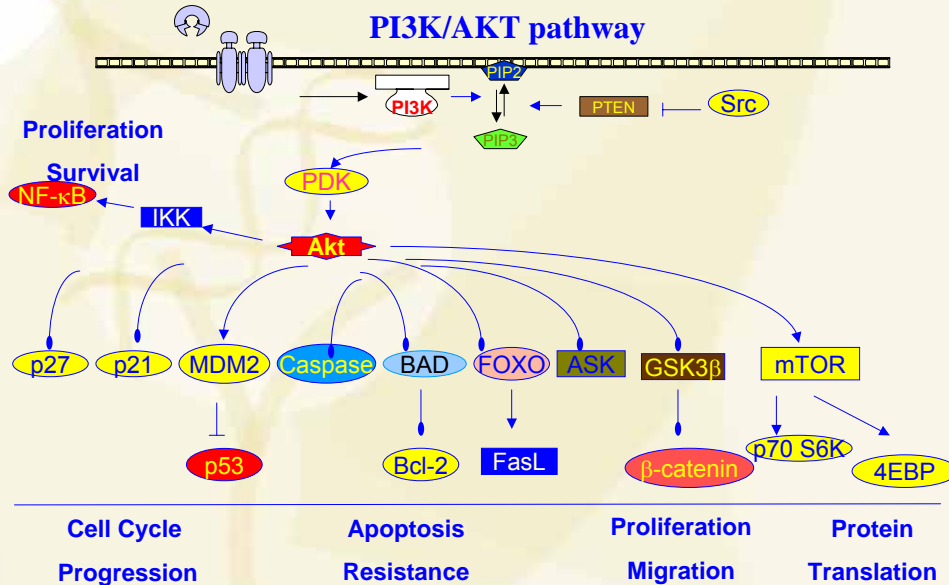


....“Cancer drugs are increasingly designed to target specific cell-signalling pathways...”

....“Pathways can be activated at different points []. If a factor at the top of a signalling cascade is unaffected, for instance, one cannot assume that the pathway is not involved, as a factor further downstream might have been activated”...

Nature 439, 274-275 (19 January 2006)

Pathway branching



Requirements for Pathway Proteomics Approaches

- Signaling is highly dynamic
 - protein phosphorylations in seconds/minutes
 - protein synthesis/degradation in hours/days
 - extensive sampling required to obtain time resolution
- Many signals are post-translational modifications
 - issue for classical MS-based methods
- Highly parallel measurements of analytes is important
 - a thorough pathway profile can easily comprise 100 or more elements
- Conclusion:
an array-based solution will provide the required scalability and throughput

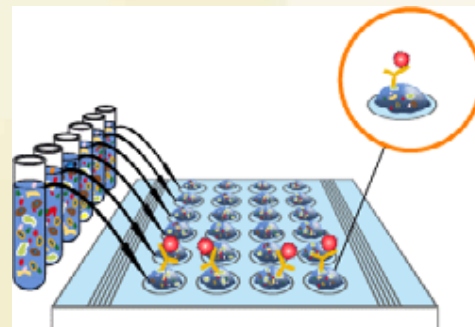
Protein Microarrays – Two Formats

Forward Protein Arrays



- Array of target-specific capture molecules (e.g. antibodies)
- One sample measured per array

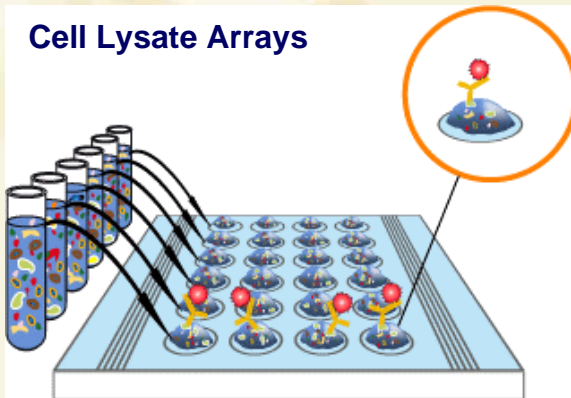
Reverse Protein Arrays



- Array of samples on chip
- One analyte measured per array

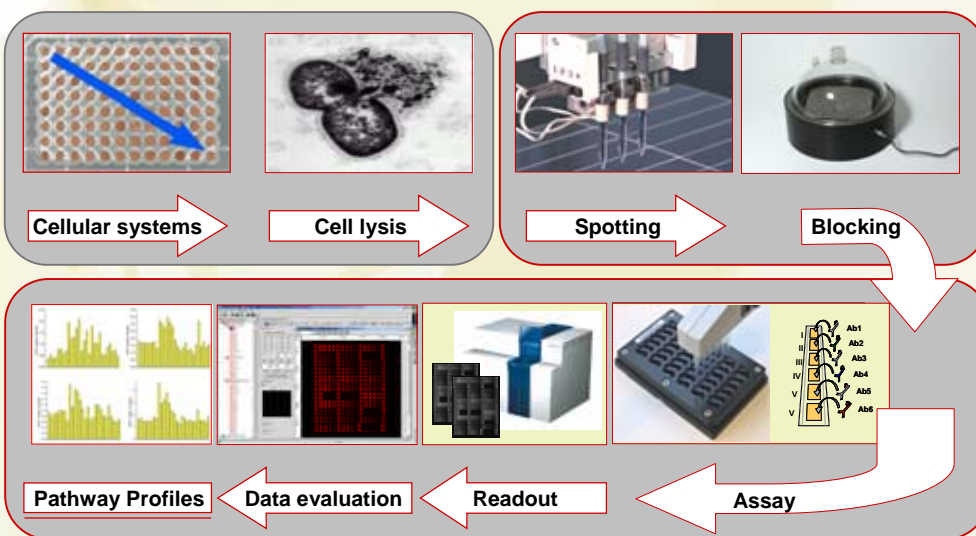
Reverse Protein Microarrays

Cell Lysate Arrays

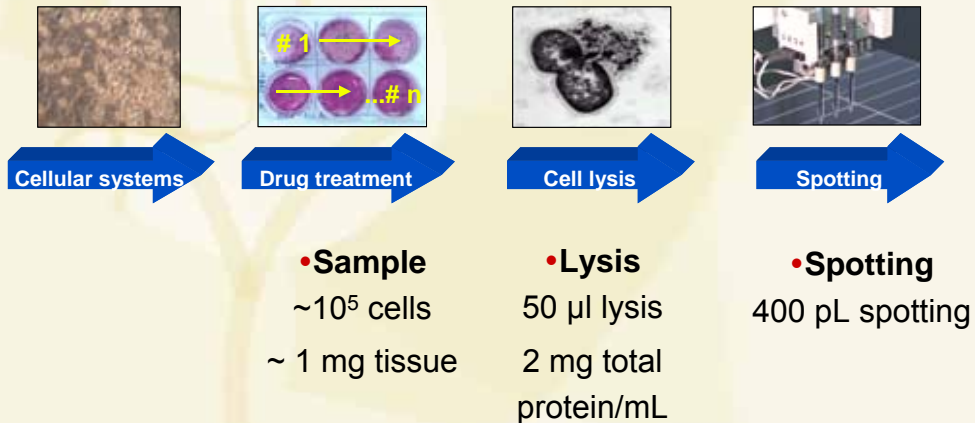


- Array of samples
- Sample volume 400 pL
- Specific detection with target-specific antibodies
- One Ab per target /array
- High flexibility in study design – minimum assay development effort
- Sample volume is never a bottle neck to measure multiple analytes

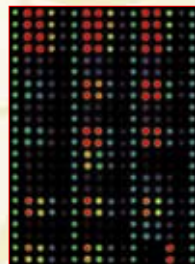
ZeptoMARK Reverse Arrays – From Cells to Protein Profiles



Zeptosens Reverse Arrays – From Cells to Protein Profiles



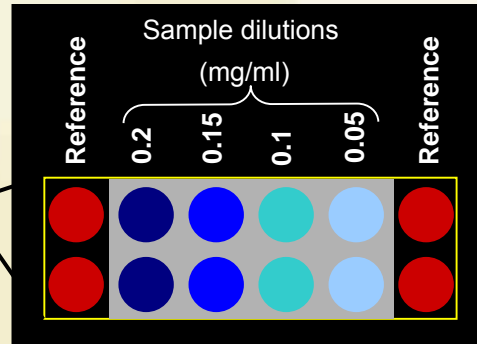
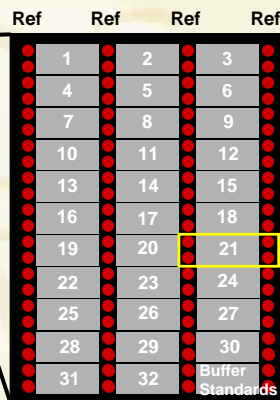
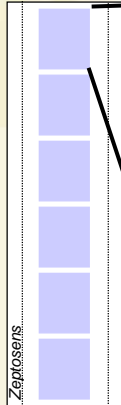
Spotting: Reproducibility & Quality



- 400pL sample volume
- Non-contact ink-jet spotting technology
- Up to 256 lysates/array or 1536 lysates/chip
- Reproducibilities of mean spot signals: CV's ≤ 2%

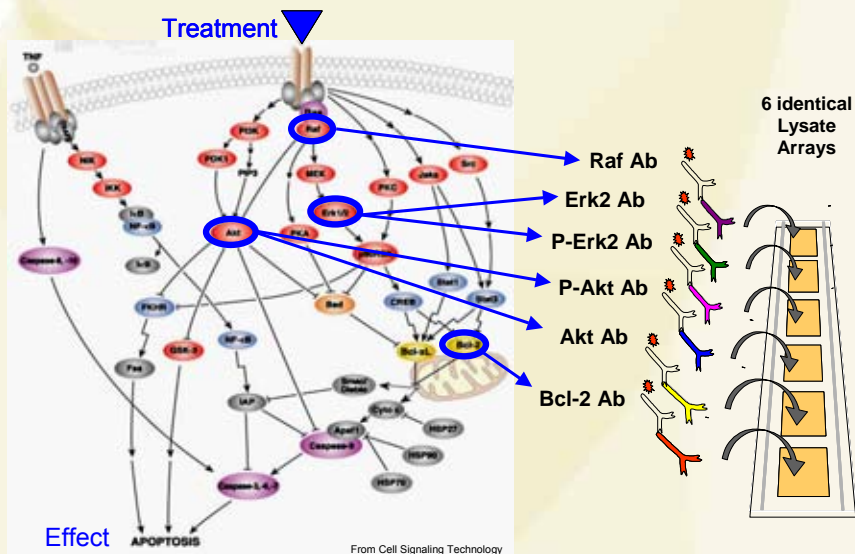
Chip and Array Layout

6 Arrays / Chip

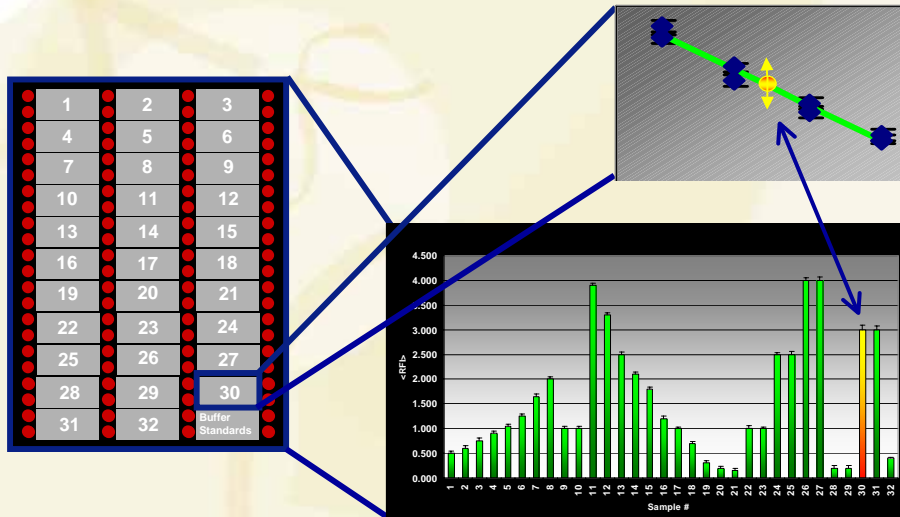


- (Ref) reference spots containing constant fluorescence
- 32 up to **32 samples** (4 dilutions, duplicates) **per array**
- up to **192 samples** (4 dilutions, duplicates) **per chip**

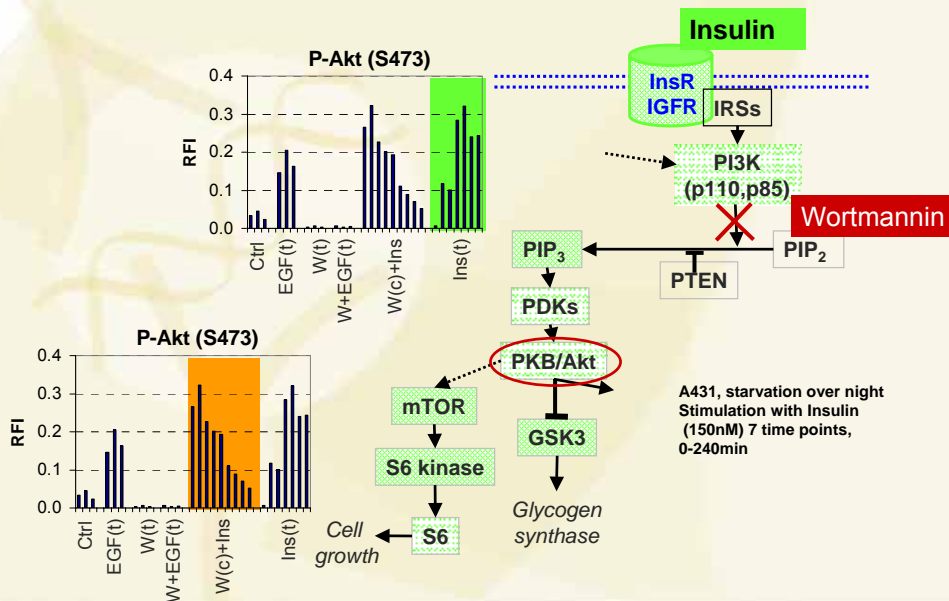
Highly Parallel Monitoring of Signaling Events



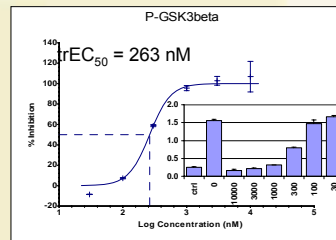
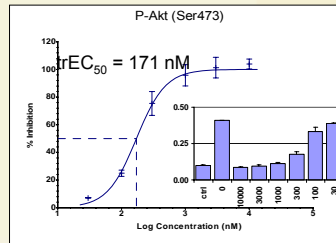
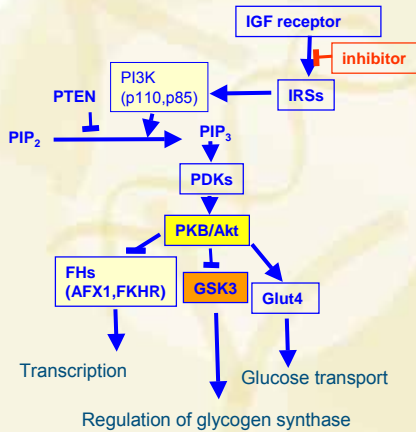
The System Response Profile



Stimulation with Insulin – Inhibition with Wortmannin

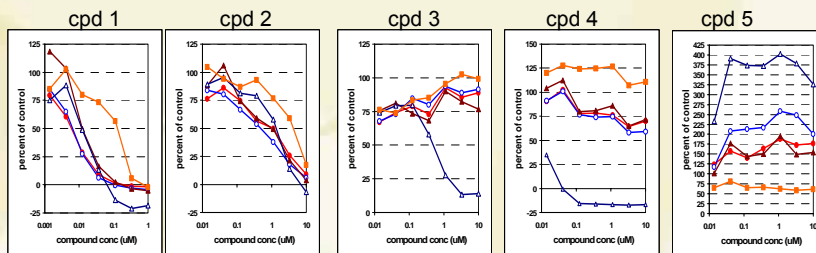


Compound Profiling using Reverse Protein Arrays



“trEC50” (transduced EC50) curves of an IGF1R inhibitor at different pathway nodes

Effects on EGFR signaling in A431 cells + EGF



	cpd 1	cpd 2	cpd 3	cpd 4	cpd 5
P-EGFR	0.006	1.038	>10	>10	Stimul.
P-STAT3	0.006	0.475	>10	>10	Stimul.
P-PLCg	0.012	1.048	>10	>10	Stimul.
P-ERK	0.129	4.243	>10	>10	plateau 60-70% of ctrl
P-AKT	0.012	1.349	0.493	<0.014	Stimul.

inhibitor of: EGFR, EGFR, PI3K, PI3K, MEK

Detection and Monitoring of Biomarkers

Pathway Atlas Disease Model versus Wild-type

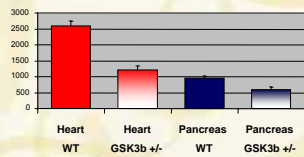


GSK-3b +/-
 heart and pancreas

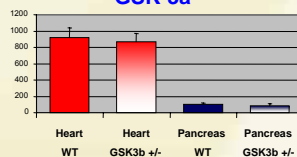


GSK-3b +/-
 heart and pancreas

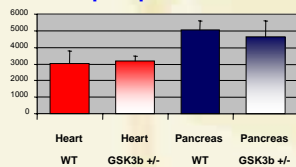
GSK-3b



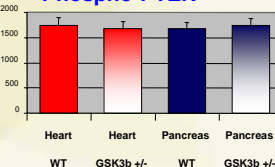
GSK-3a



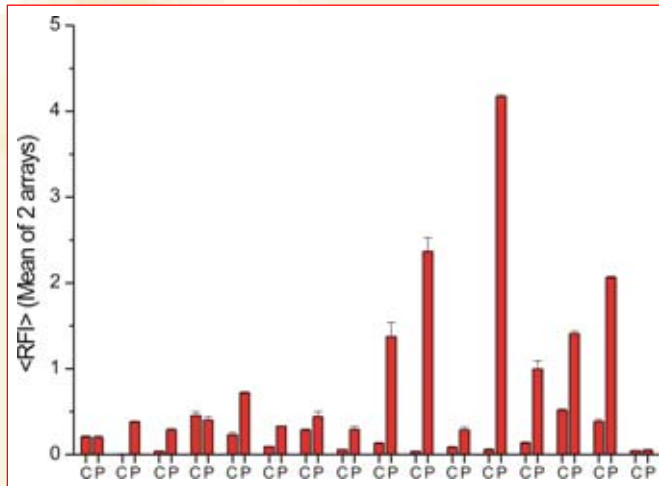
Phospho-p44/42 MAPK



Phospho-PTEN



Liver Cirrhosis Biomarker Evaluation in Urine



Comparison of 16 pairs of sex and age matched Controls (C) / Patients (P)

10 liver cirrhosis candidate markers were measured whereas results for one are illustrated here

Protein concentrations between 2.5 to 10 $\mu\text{g/ml}$
No concentration step required

In collaboration with Fernando Corrales, CIM

Microarrays for Highly Efficient Reconnaissance of Complex Signaling Network Systems

