

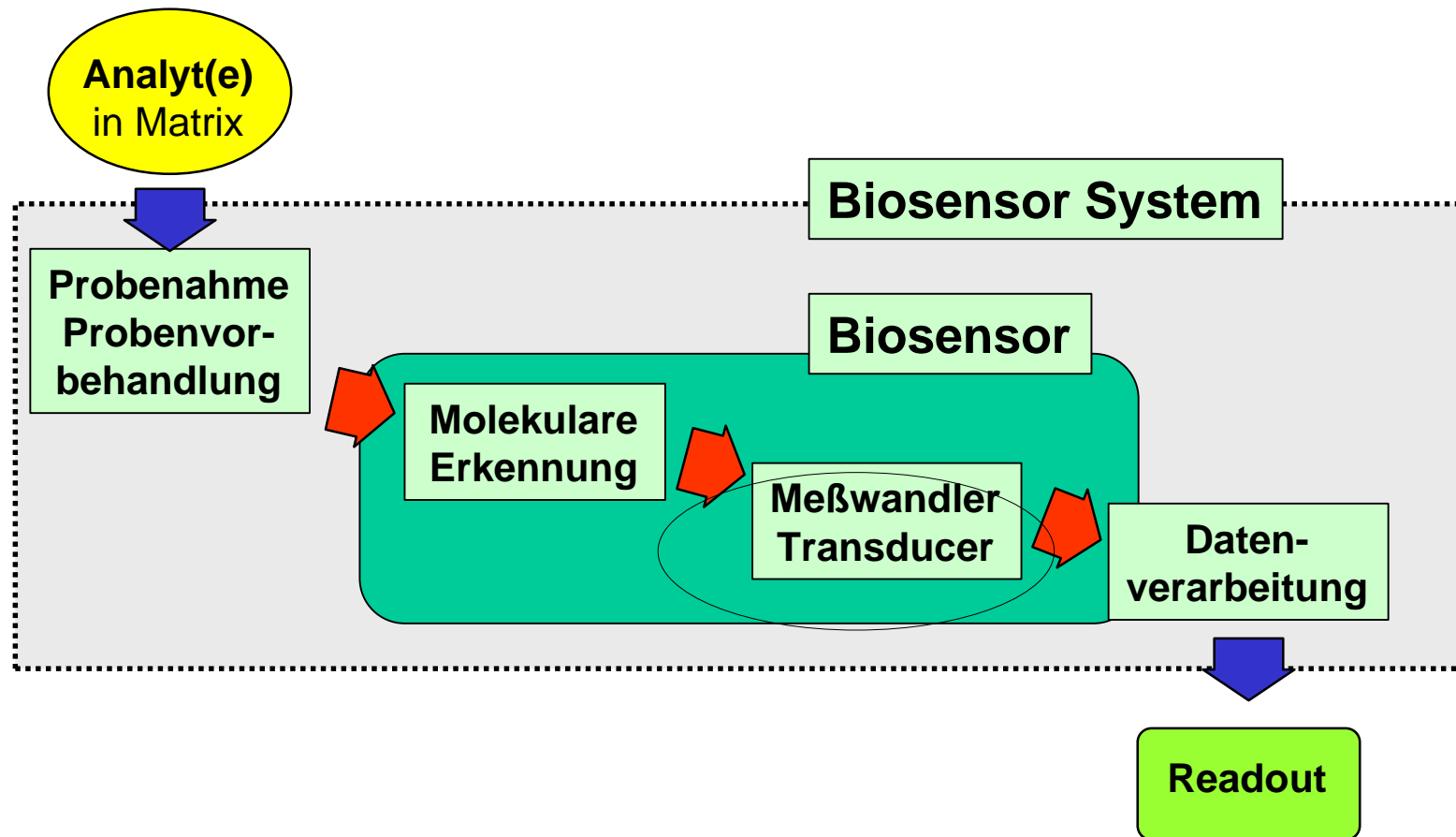
Biosensorik und Biochips

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Überblick

- Biosensor = Chemosensor mit biologischen Komponenten (s. Vorl. 9)
- Chemosensorik-Grundlagen (s. Vorl. 9)
- Biologische Erkennungsprozesse und -moleküle (s. Vorl. 9)
- Transducerprinzipien und Beispiele für Biosensoren
- Biochips: technische Prinzipien, Beispiele und Anwendungen

Funktionelle Schichten eines (Bio-)Chemosensors



Biosensorik - Überblick

- **Transducerprinzipien**

- mechanisch (piezo, akusto-gravimetrisch): QCM, FPW, SAW, Cantilever
- thermisch (kalorimetrisch)
- elektrisch (Widerstand, Impedanz; elektro-chemisch)
- optisch (Brechungsindex, Absorption, Lumineszenz) - (s. Vorl. 10 - 12)

- **Beispiele für Biosensoren**

- Akustogravimetrischer Bakteriensensor
- Kalorimetr. Biosensoren: Enzym-Thermistor-Sensor
- Amperometrische enzymatische Glucosesensoren
- Opt. Sensoren: evaneszente Welle, Fasersensoren, SPR, TIRF etc (s. Vorl. 10 - 12) - „molecular beacon“

- **Beispiele für Biochips**

- DNA-Microarrays

Der Biosensor-Steckbrief

- Funktion
 - Analyt, Messbereich, Genauigkeit
 - Matrix, Störgrößen
- Prinzipien
 - Molekulare Erkennung
 - Transducer-Prinzip
- System
 - Probenvorbereitung
 - Reagentienzugabe
 - Auslesetechnik, -gerät
 - Systemintegration (auch IT: Bioinformatik)
- Entwicklung
 - Erforschung (proof-of-principles)
 - Prototypen
 - marktfähiges Produkt, Marktetablierung

Transducerprinzip und Meßgrößen

Informationsübertragungskette - Integration

Transducerprinzip

Akusto-gravimetrisch

Thermisch

Elektrisch

Elektro-chemisch

Optisch

Primäre Meßgrößen

Masse -> Schwingungsfrequenz

Wärmemenge -> Temperatur

Impedanz (el. Leitfähigkeit)

Spannung, Strom

Brechungsindex, Absorption

Emission (Fluoreszenz) -

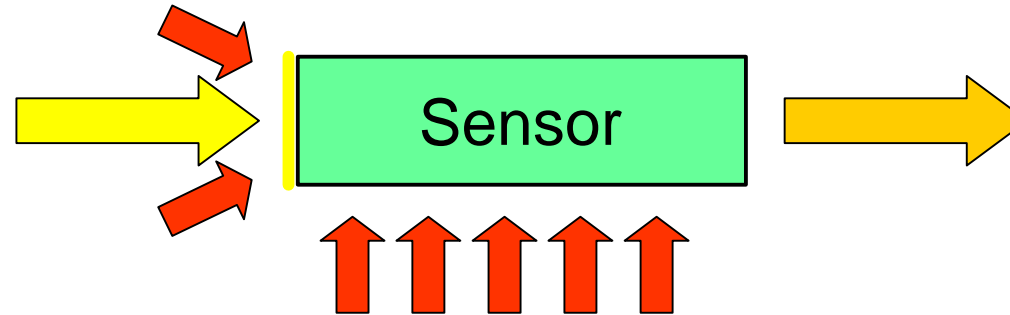
Lichtintensität (Photometrie)

Finale Meßgröße

el. Spannung (Strom)

Ziel: Markierungsfreie (label-free) Detektion des Analyten

Die integrale Sensorfunktionsgleichung



$$\text{Sensorsignal} = f(\text{Nutzgröße}, \text{Störgrößen})$$

- **Nutzgröße:** Analytkonzentration
- **Störgrößen:**
 - chemisch: störende Stoffe
 - physikalisch: mechanisch, thermisch, elektrisch, optisch
 - Zeit: Alterung, Biofouling bei implantierten Sensoren
- **Reversibilität**
- **zeitl. Dynamik**

chem. Signal

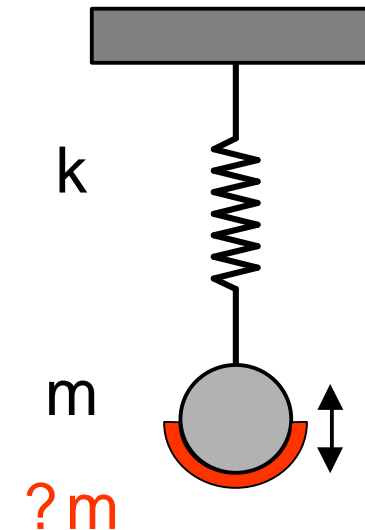


elektr. Signal



Akusto-gravimetrischer Sensor - Prinzip

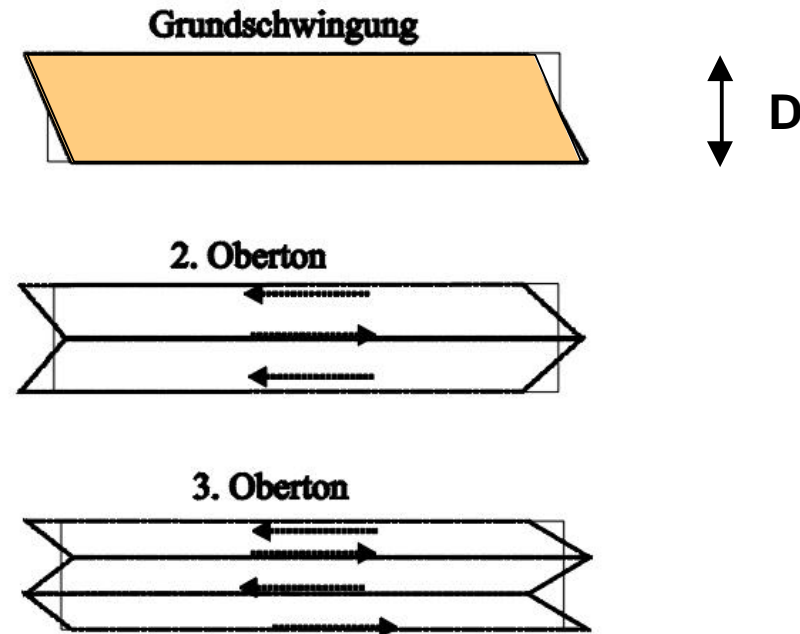
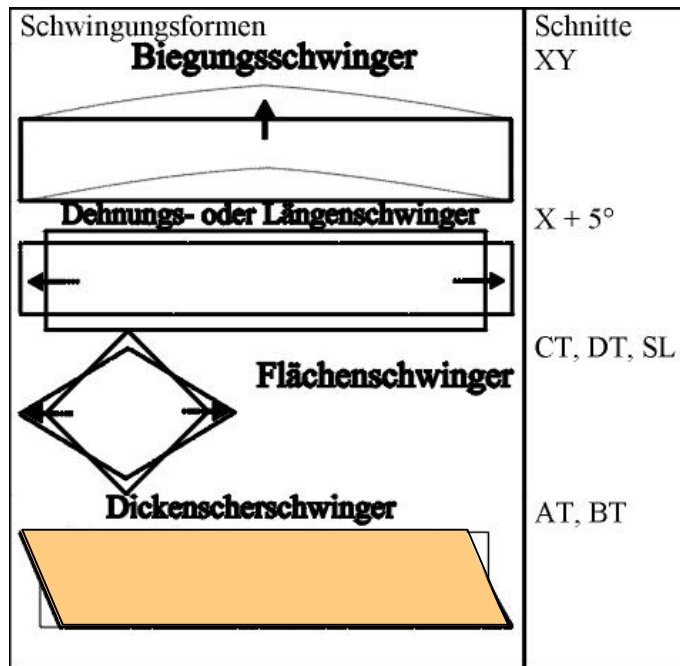
- Mechanischer (akustischer) Resonator / Welle
 - elastischer Körper
 - effektive Masse m , Rückstellkonstante k
 - Eigenfrequenz f
 - Frequenzänderung durch $? m$
 - Form und Schwingungsmode
Volumen, Oberfläche, Biegeplatte
- Elektro-akustische Wandler
 - Piezo-Effekt
 - elektrostatisch
 - elektromagnetisch
 - optisch (-elektrisch)
- Beispiele (nach Schwingungsmoden)
 - Quarzmikrowaage QCM (Volumenschwinger)
 - Oberflächenwellensensor SAW
 - Biegeplattenwellensensor FPW
 - Cantilever (Biegebalken)



$$f = \frac{1}{2p} \sqrt{\frac{k}{m}}$$

$$\frac{\Delta f}{f} = -\frac{1}{2} \frac{\Delta m}{m}$$

QCM - Quartz Crystal Microbalance Schwingungsformen (Moden)



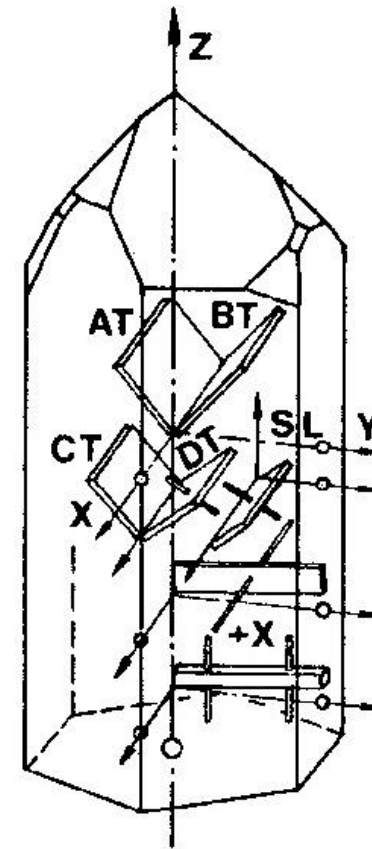
**Für Biosensoren am gebräuchlichsten:
Dickenschwinger (für Quarz, AT-Schnitt)**

$$f \text{ (MHz)} \gg 1670 / D \text{ (}\mu\text{m)}$$

f (MHz)	D (μm)
10	167
35	48

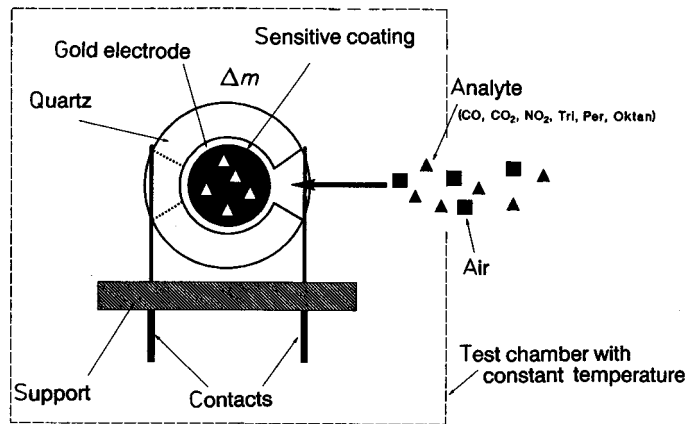
QCM - Quartz Crystal Microbalance

Schnittrichtungen der Quarze



QCM - Quartz Crystal Microbalance

Massensensor mit Schwingquarz (10 MHz)

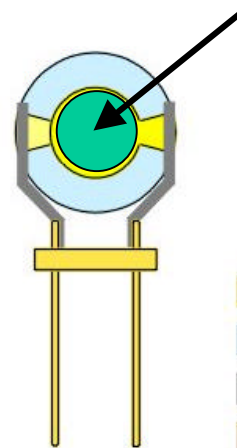


$$\Delta f_F = - \frac{f_0^2}{N \rho_{\text{Quarz}} F} m_F$$

- f_0 = Resonanzfrequenz,
- N = Frequenzkonstante
= 167000 Hz cm
- ρ_{Quarz} = Dichte von Quarz
= 2,658 g/cm³
- F = Elektrodenfläche
- m_F = Masse der Fremdschicht

(Sauerbrey 1959)

Biologische Beschichtung



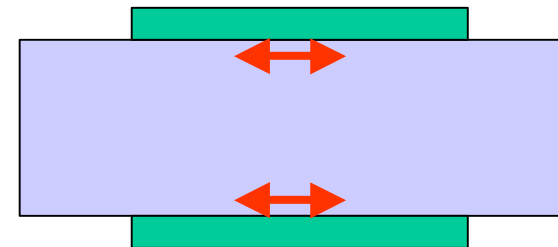
- = Goldelektrode
- = Quarkscheibe Dicke: 168µm
- = Kontaktierung des Quarzes
- = Halterung HC 49

QCM - Zusammenfassung

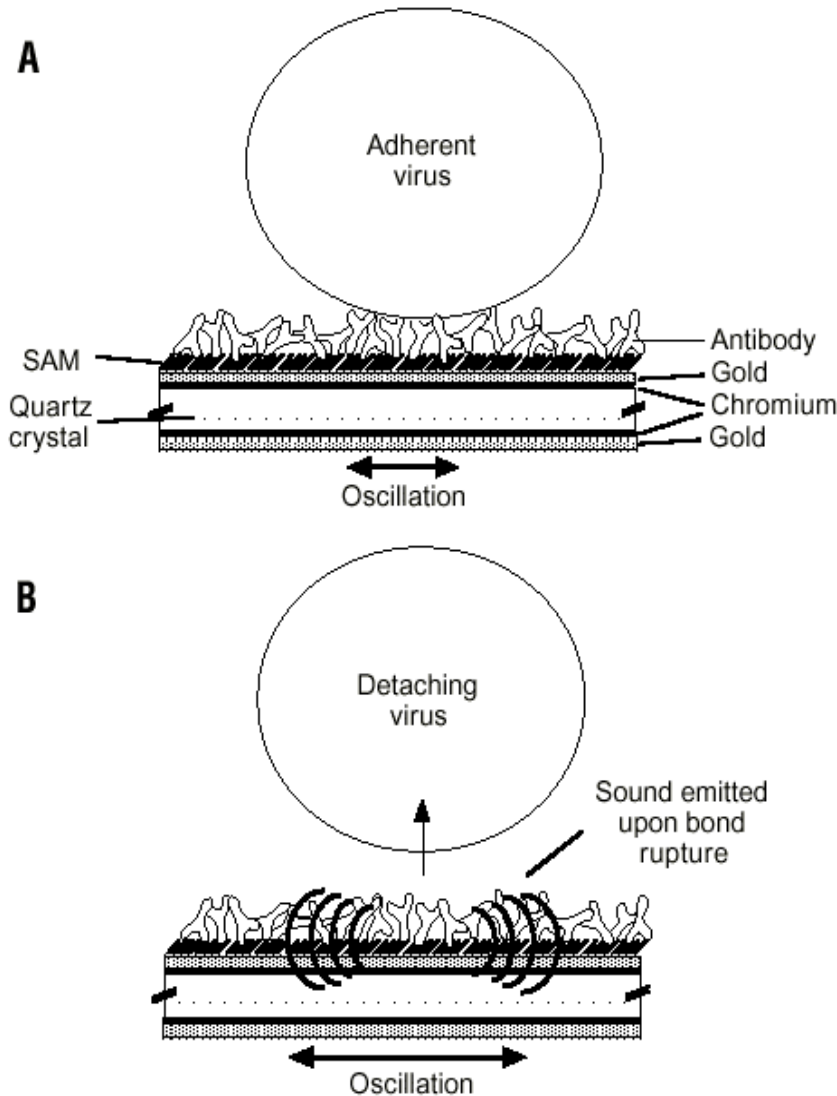
- Massensensitiver Volumenschwinger (BAW)
- Frequenz 5 - 35 MHz, dünne Quarzplättchen (AT-Schnitt)
- Sensitivität (in Gas) $4,4 \times 10^{-9} \text{ g/cm}^2$ (bei 10 MHz)

$$S_m = \lim_{\Delta m \rightarrow 0} \frac{1}{f_0} \frac{\Delta f}{\Delta m} = \frac{1}{f_0} \frac{\partial f}{\partial m} = \frac{-2}{\rho \lambda} = \frac{-1}{\rho d}$$

- Art der Schicht
 - starr -> ?f
 - viskoelastisch -> ?f, ?Q (Dämpfung)
- Schlechte Empfindlichkeit in Flüssigkeiten (Scherschwingung, Flüssigkeit dämpft)



QCM - Rupture event scanning (REVS)

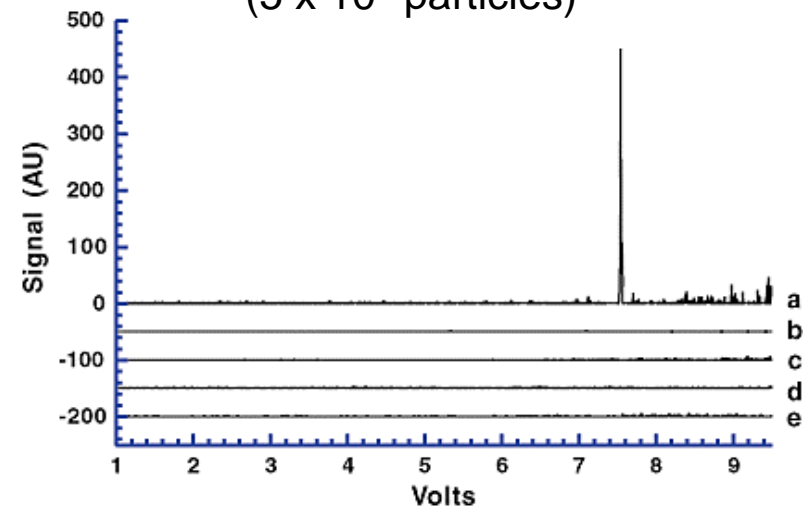


Direct and sensitive detection of a human virus by rupture event scanning

Matthew A. Cooper *et al.*

Nature Biotechnology 19, 833 - 837 (2001)

Sound signal
(5×10^5 particles)

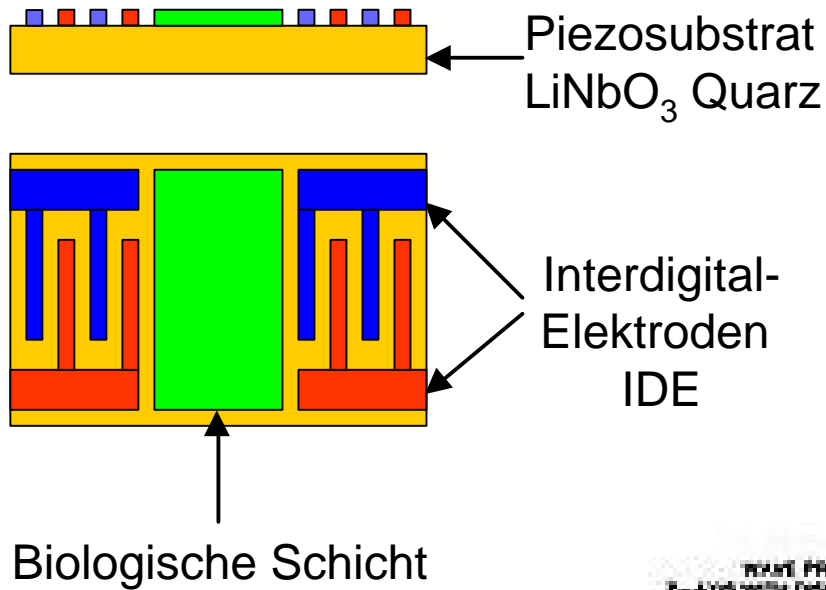


Limit of detection
10 - 100 virus particles

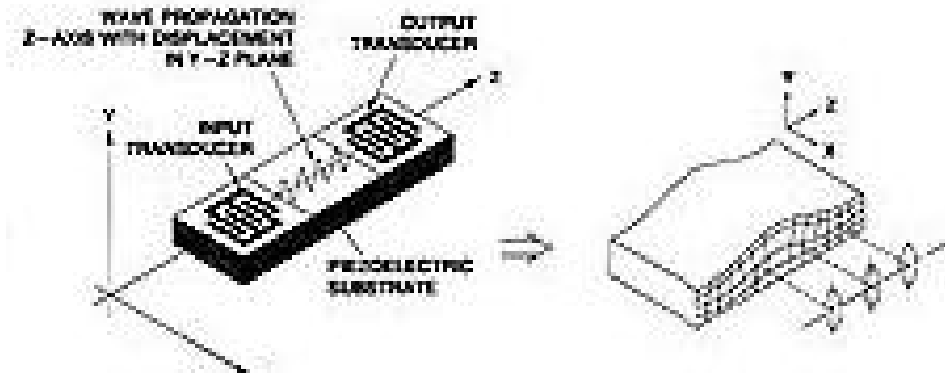
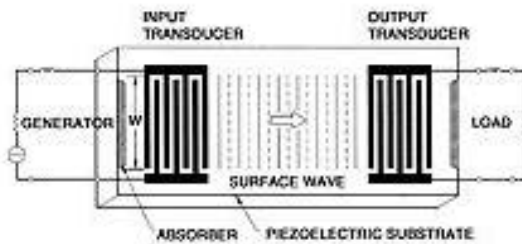
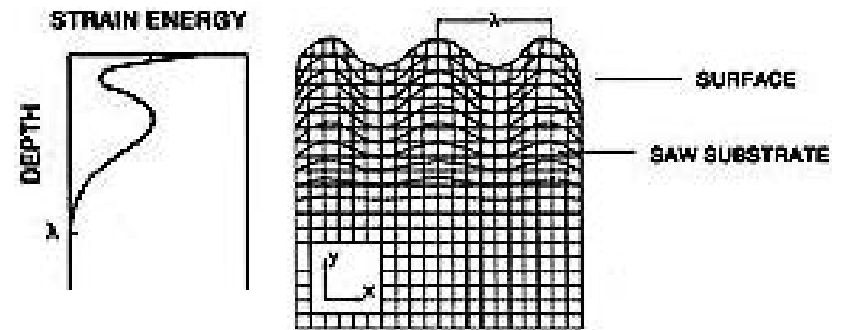
Surface Acoustic Wave (SAW) Sensor

Oberflächenwellen-Sensor

Prinzip



Schwingungsmode Rayleigh (1885)

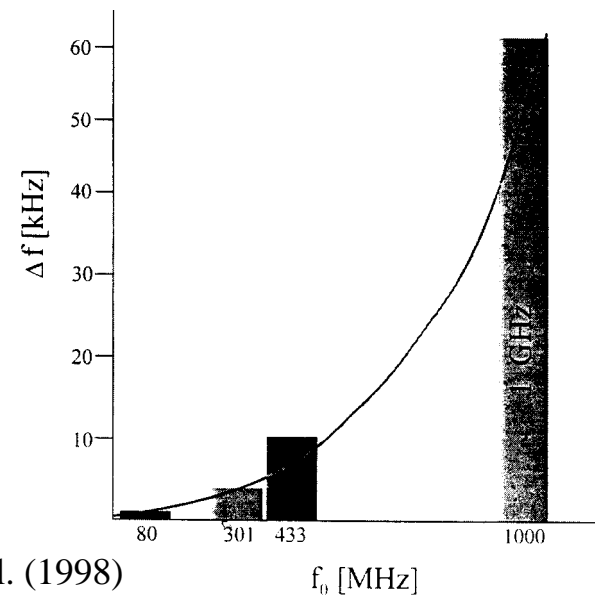


SAW-Sensor

- Oberflächenwelle
- Frequenz 100 - 1500 MHz, Amplitude ca. 1 nm
- Empfindlichkeit (unabh. von Plättchendicke)

$$S_m = \lim_{\Delta m \rightarrow 0} \frac{1}{f_0} \frac{\Delta f}{\Delta m} = \frac{1}{f_0} \frac{\partial f}{\partial m} = \frac{-2}{\rho \lambda} = \frac{-1}{\rho a}$$

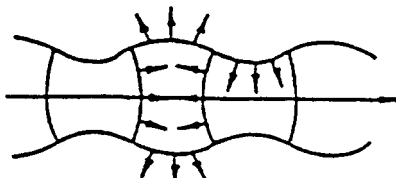
- Sehr empfindlich für Gase
Luftmonitoring (organ. Dämpfe,
Schadstoffe, chem. Kampfstoffe)
- Beachte Signal/Rauschen!
z. B. $S \sim f^2$, $R \sim f \rightarrow S/R \sim f$



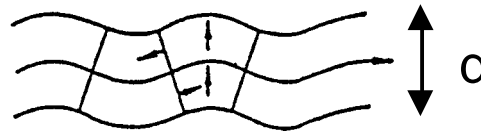
Dickert et al. (1998)

Biegeplattenwellen-Sensor Flexural Plate Wave (FPW) Sensor

Plattendicke $d \ll l$



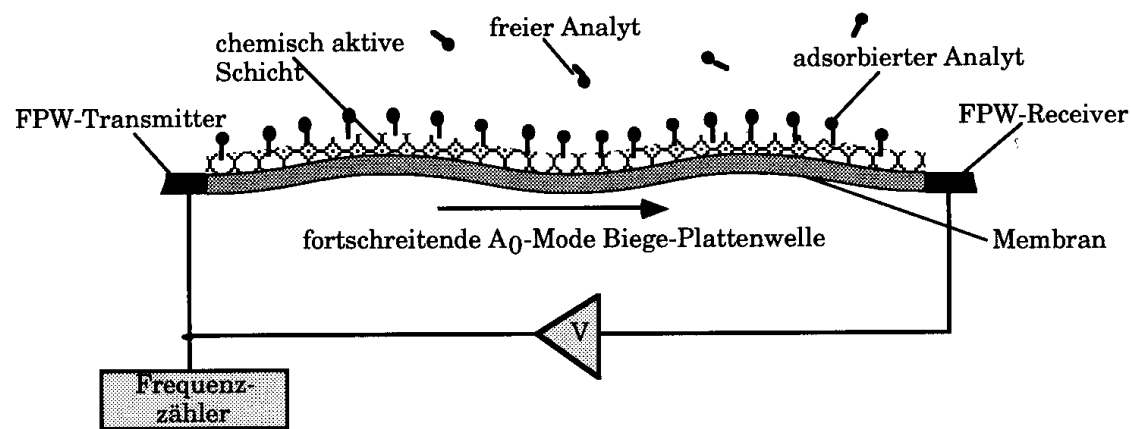
S-Mode
symmetrisch



A-Mode
asymmetrisch

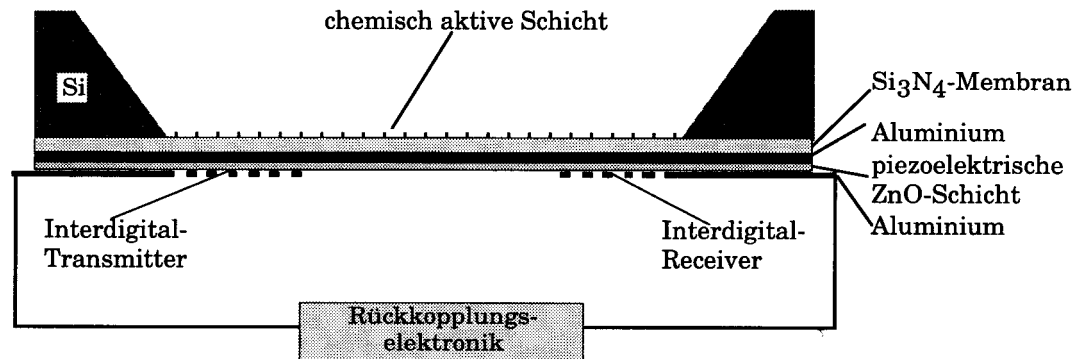
Empfindlichkeit

$$S_m = \frac{-1}{2\rho d}$$



FPW - Sensor

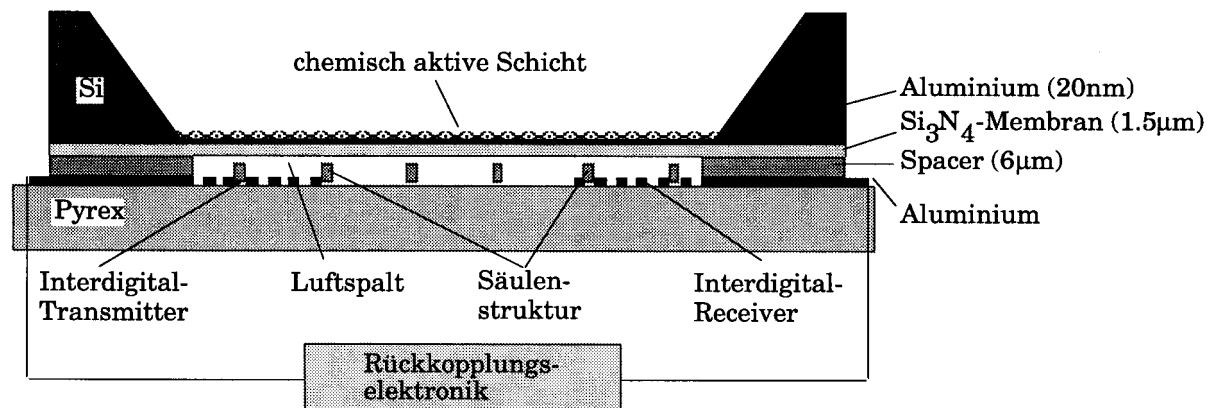
Piezo-Wandler



Empfindlichkeit

$$S_m = \frac{-1}{2\rho d}$$

Elektrostatischer Wandler



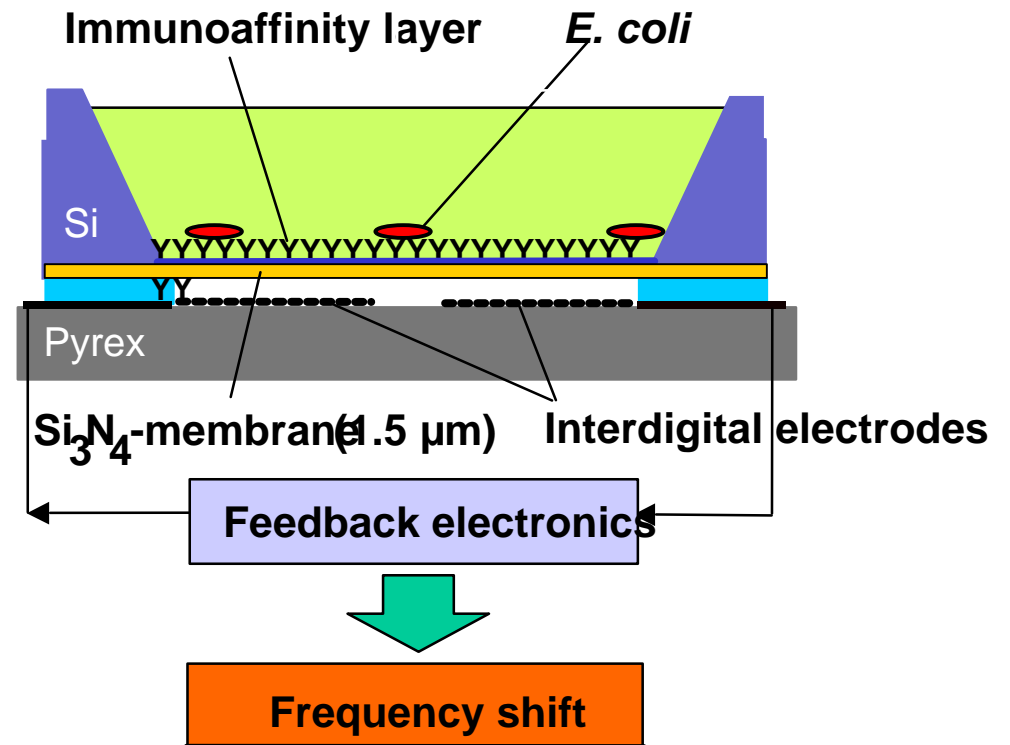
Development of a Sensitive Acousto-Gravimetric Immunoaffinity Biosensor for *E. coli* Bacteria

Hans Heinrich Ruf, Jae-Chul Pyun, Hansjörg Beutel, Jörg-Uwe Meyer

**Abteilung Sensorsysteme / Mikrosysteme
Fraunhofer Institut für Biomedizinische Technik (IBMT)
D-66386 St. Ingbert, Germany**

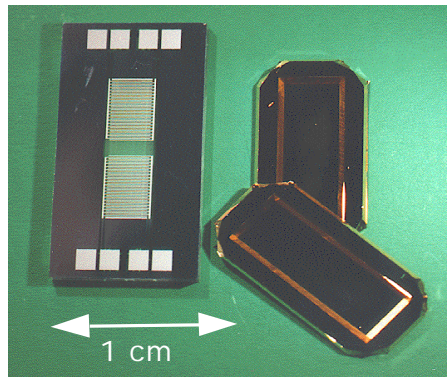
Approach:

- Biosensor
- Acoustogravimetric transducer with immunoaffinity molecular recognition layer
- Flexural plate wave (FPW) transducer:
 - FPW on thin membrane (Lamb wave in A_0 mode)
 - High sensitivity in fluids
 - Thick sensitive layer (evanescent acoustic wave)

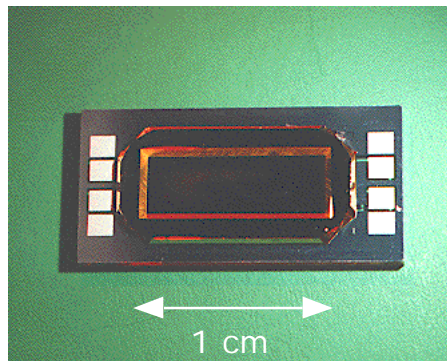


Flexural Plate Wave (FPW) Transducer Microfabricated Transducers

2nd Generation

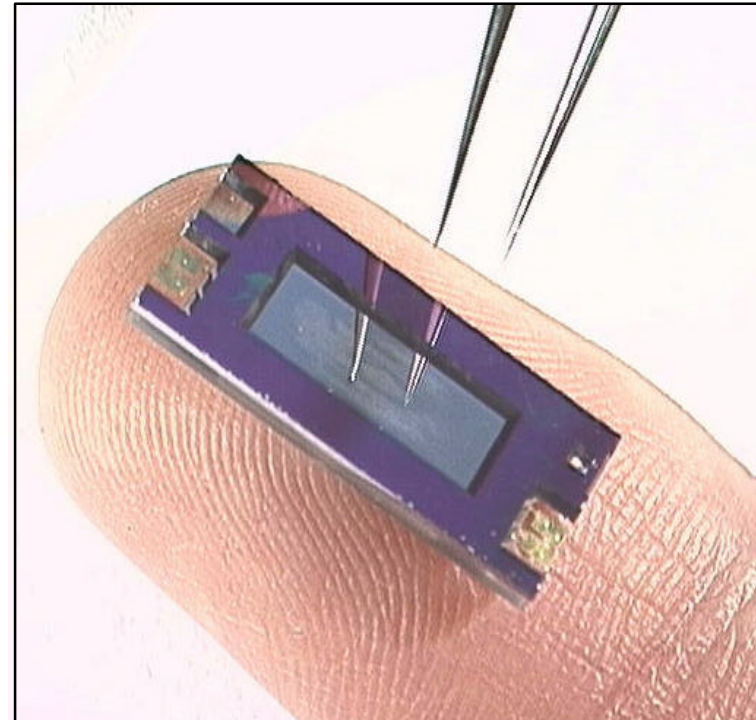


Components



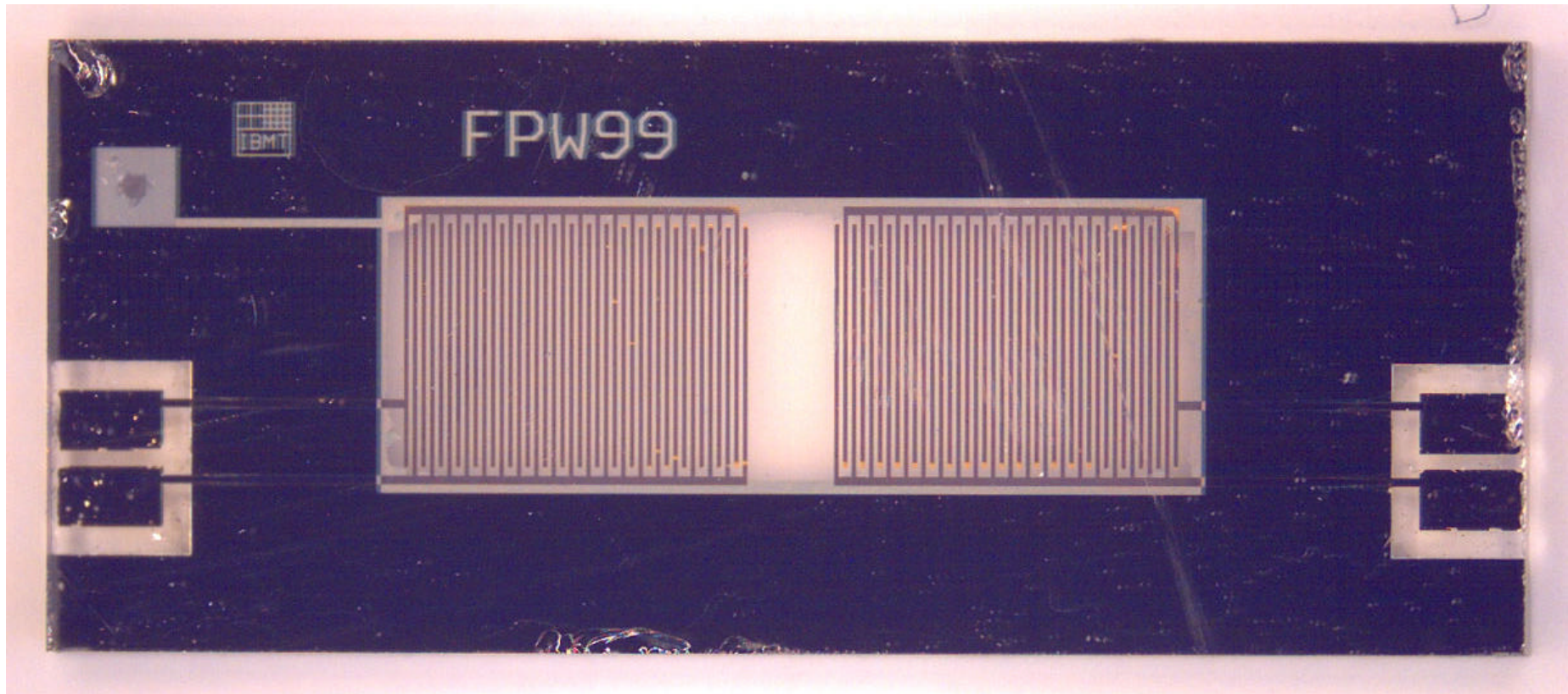
Assembled by gluing

3rd Generation



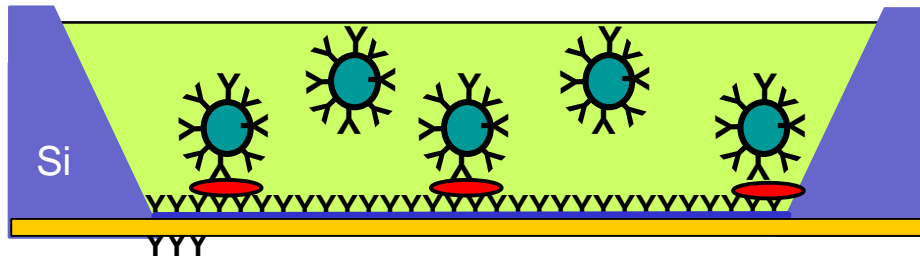
Assembled by anodic bonding

FPW-Sensor - Interdigitalelektroden



40 Finger, Periode 200 μm , aktive Fläche 9 x 3 mm

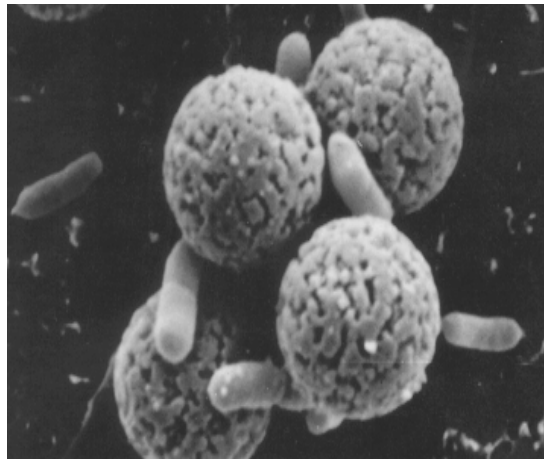
Approach for Amplification Procedure



Idea

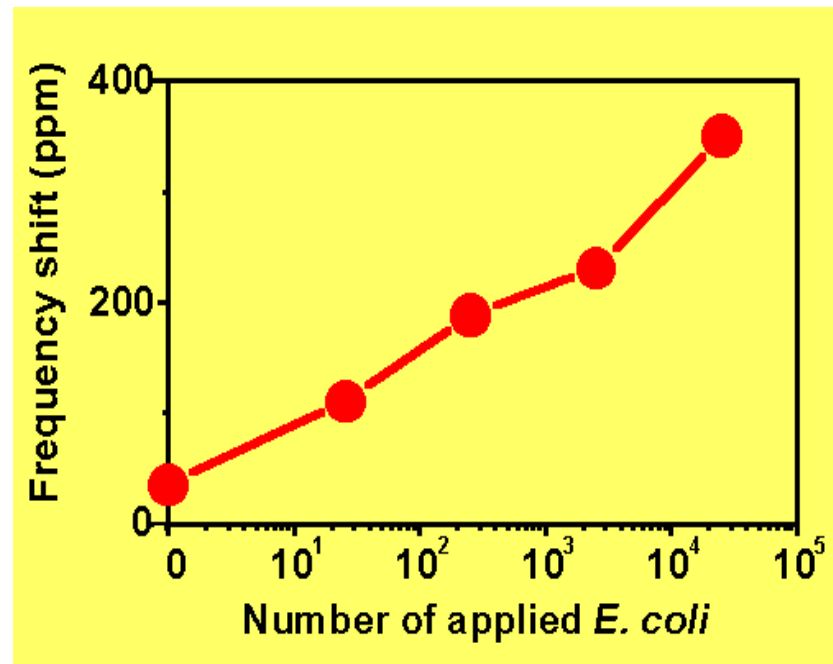


Complex of *E. coli* with microspheres increases effective mass of bacteria (0.3 pg/*E. coli* ->3.5 pg/microsphere).



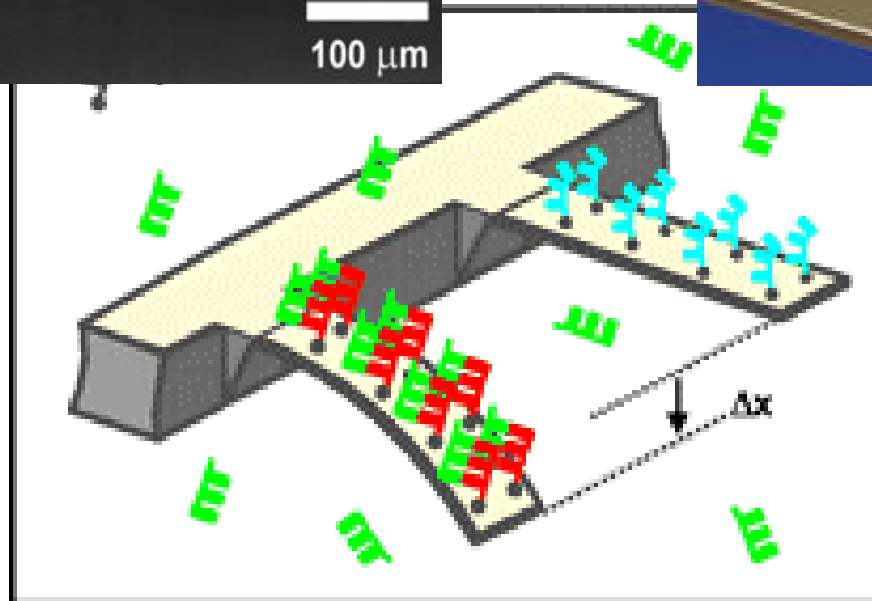
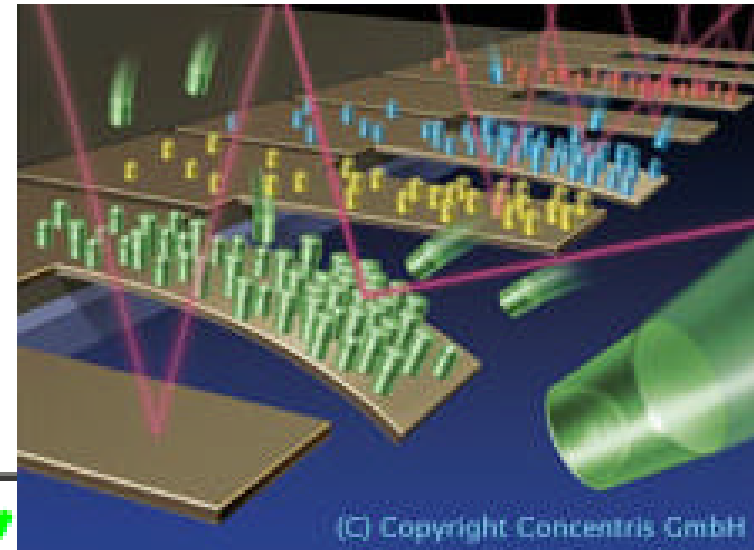
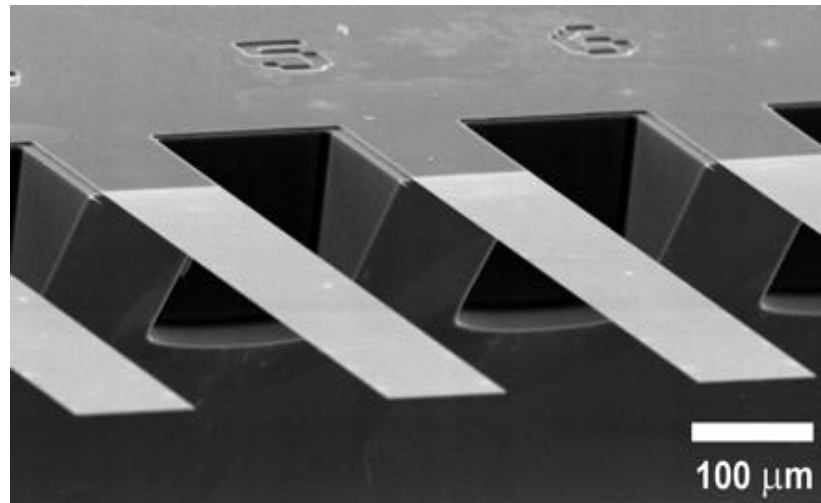
Complex of *E. coli* with microspheres (REM picture from Dynal A. S.)

Detection of *E. coli* with FPW Biosensor and Optimized Procedure Using Sampling and Amplification by Microspheres

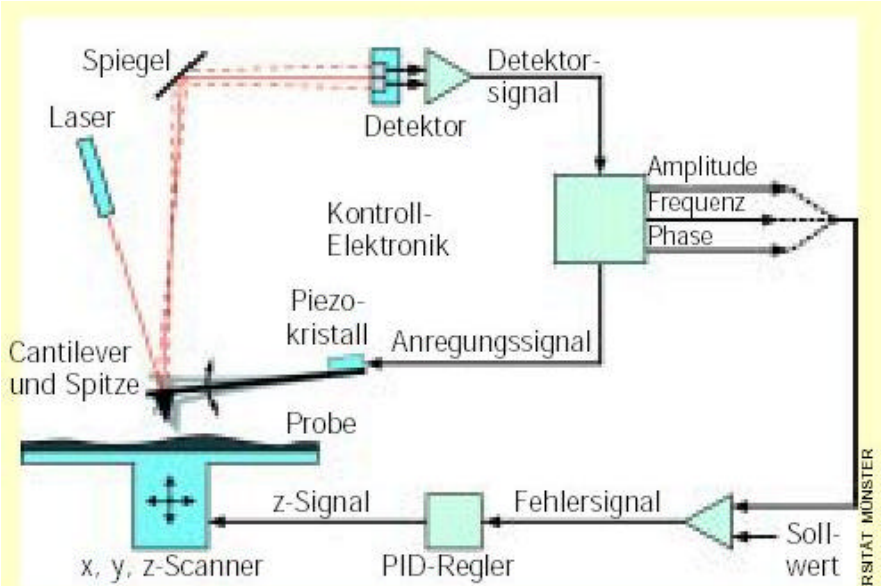


Detection limit	100 <i>E. coli</i>/ml
Amplification	100-fold

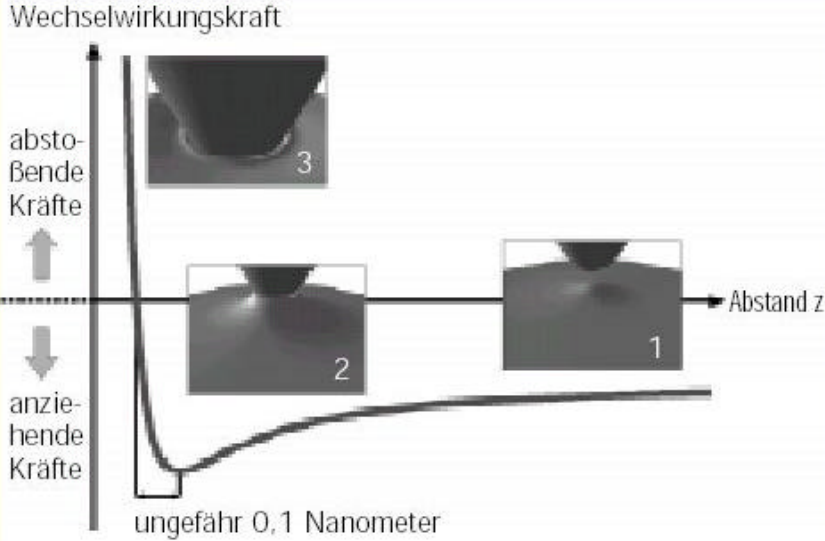
Cantilever Sensor - Prinzip „Biegebalken“



Cantilever AFM



Schematischer Aufbau eines Rasterkraftmikroskops



BEIDE ABBILDUNGEN: PHYSIKALISCHES INSTITUT, WESTFÄLISCHE WILHELMS-UNIVERSITÄT MÜNSTER

Kommerzieller Cantilever-Sensor 1

Cantisens Research

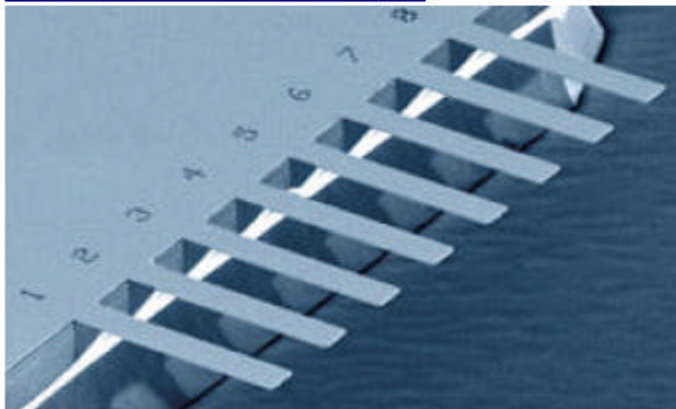


Cantisens Research is a cantilever sensor platform designed specifically for industrial application development and scientific research.

- ◆ Eight channel label-free biochemical sensing.
- ◆ Low volume liquid chamber.
- ◆ Integrated temperature control.
- ◆ Sample handling system included.

Click [here](#) for more information.

Cantilever Arrays



<http://www.concentris.ch/>

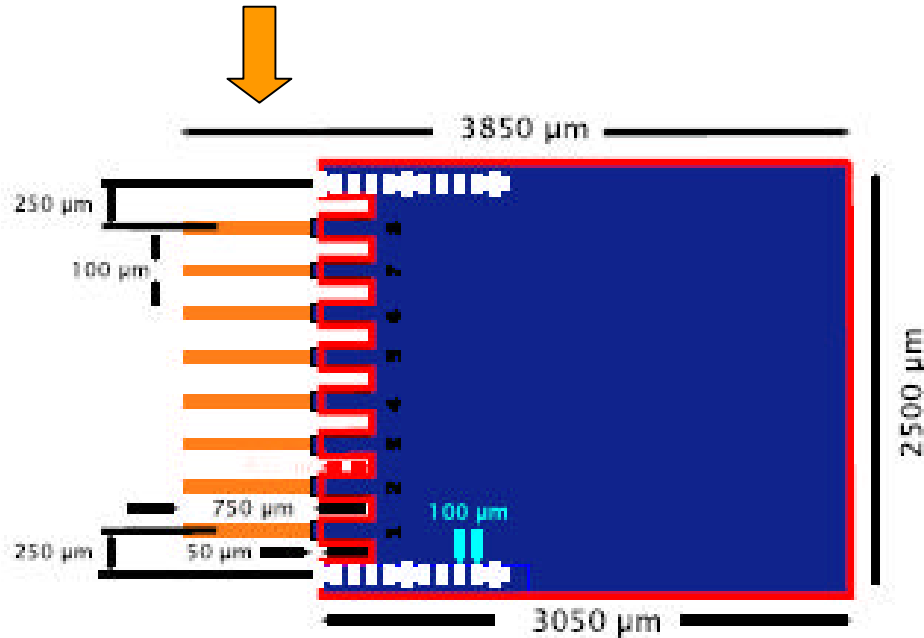
High quality cantilever arrays are now available in two different geometries.

- ◆ Eight high precision levers per array.
- ◆ Lengths of 500 μm and 750 μm .
- ◆ Thickness of 1 μm for highest sensitivity.
- ◆ Width 100 μm , pitch 250 μm .

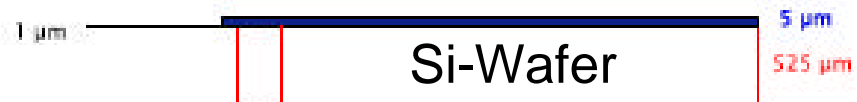
[Download](#) cantilever array specifications (PDF).
[Contact us](#) for information on how to order.

Kommerzieller Cantilever-Sensor 2

8 Cantilevers $750 (500) \times 100 \times 1 \mu\text{m}^3$



Side View of $750\mu\text{m} \times 100\mu\text{m} \times 1\mu\text{m}$ cantilever arrays



Concentris GmbH
Davidsbodenstrasse 63
CH - 4056 Basel
Switzerland

www.concentris.com

Elektrochemische Biosensoren

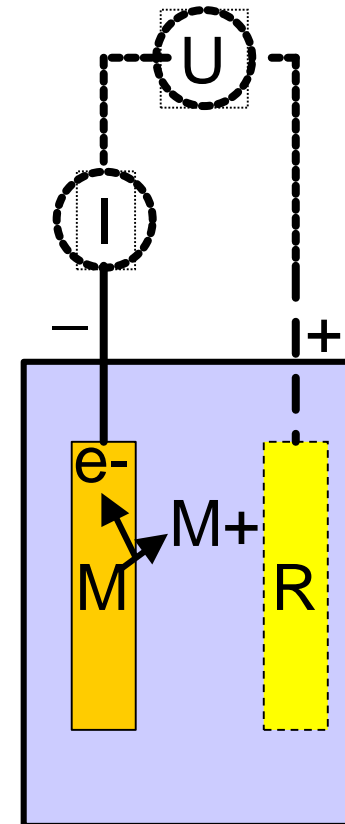
- System: Elektrode in Lösung
 - Redoxreaktion
 - Nernst'sche Gleichung
- Potentiometrie
 - Potentialmessung durch FET (ChemFET, ISFET)
- Voltammetrie
 - Reaktionen und Vorgänge an der Elektrode (Redox, Diffusion)
 - Meßprinzip $I = f(U(t))$
 - Amperometrische Biosensoren (z. B. Glucose)

Elektrode in Lösung - Elektrochem. Zelle

- Redoxreaktion $M \rightarrow M^+ + e^-$
- Im Gleichgewicht stellt sich Potential ein ($I = 0$):
 - Nernst'sche Gleichung

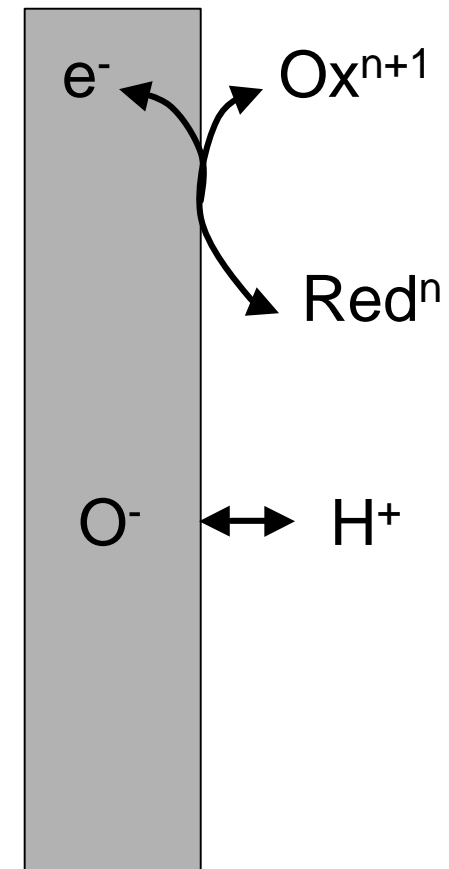
$$\Delta E = \Delta E^0 + \frac{2,303RT}{nF} \log \frac{c_{ox}}{c_{red}}$$

- “Potentiometrie”
- Bei angelegter Spannung läuft die Redoxreaktion ab \rightarrow Strom I fließt (Elektrolyse)
 - Strom hängt von Spannung und Konzentration der redox-aktiven Stoffe ab (und von Elektrodenoberfläche)
 - Bei spezifischen Bedingungen ist der Strom ein Maß für [Analyt]
 - “Amperometrie”

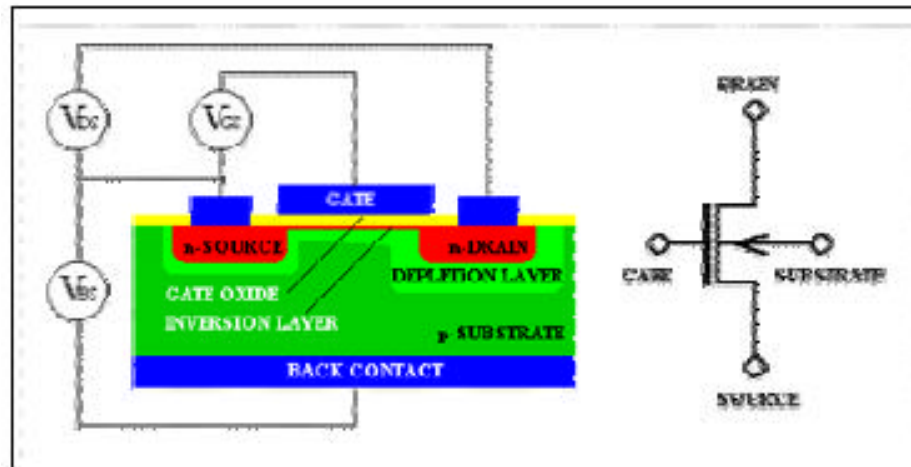


Elektrodenreaktionen

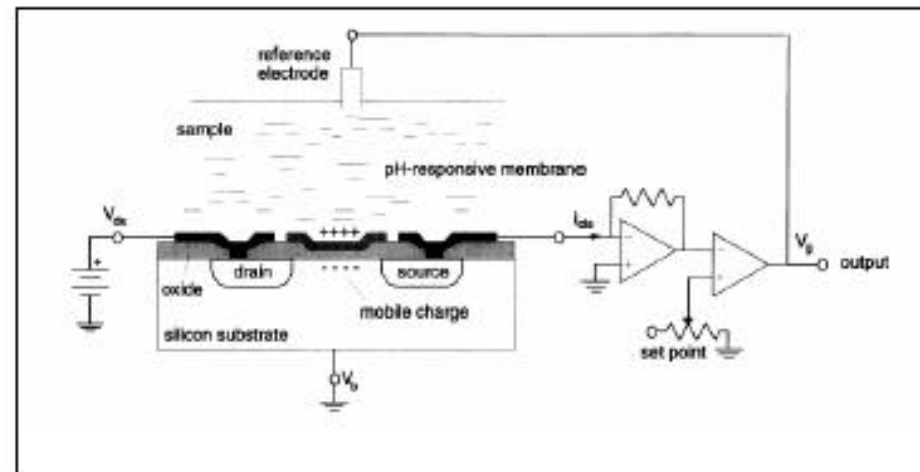
- Redoxreaktion mit Elektrode (Metall)
- Redoxreaktion an Elektrode
- Adsorption von Ionen an Gegenionen der Oberfläche (H^+ an Glas, Ionen an ionensensitiven Schichten)
- Die Reaktion mit dem schnellsten Austausch bestimmt das Potential der Elektrode



Potentiometrischer Sensor - ChemFET, ISFET

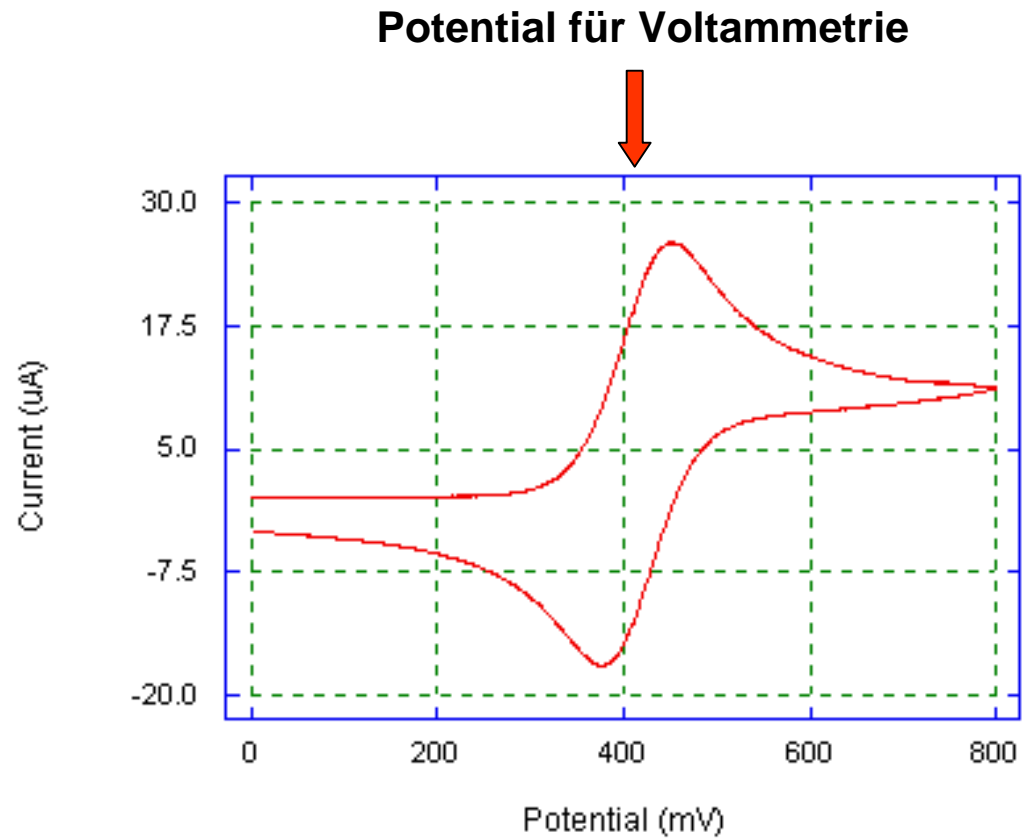
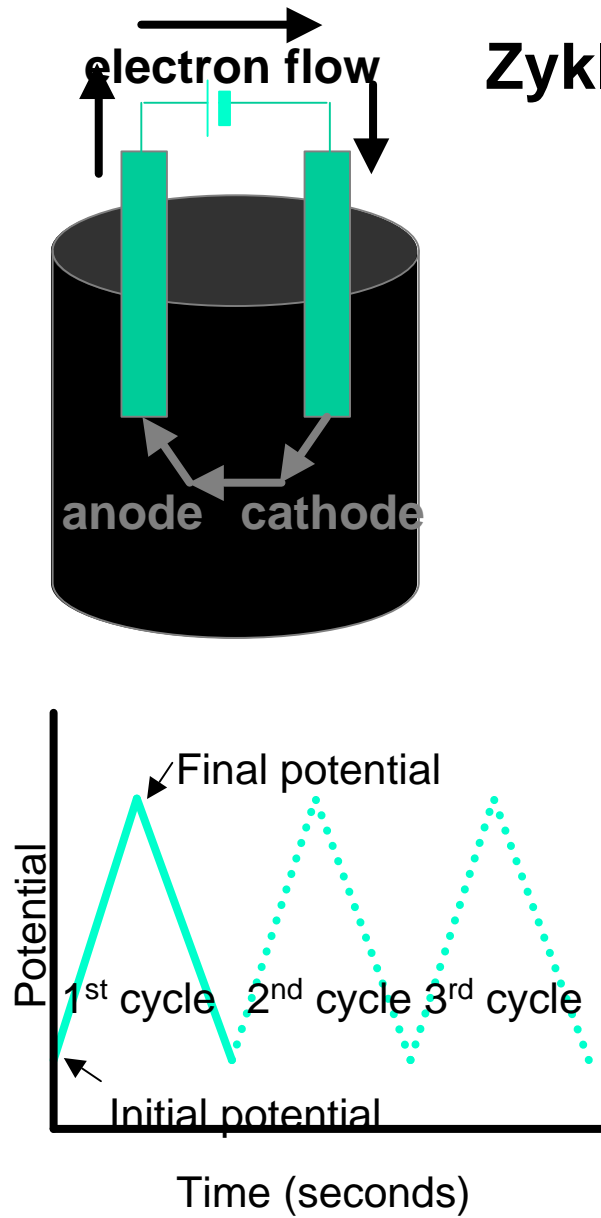


Typical layout of a FET



Ion-Sensitive Field Effect Transistor (ISFET)

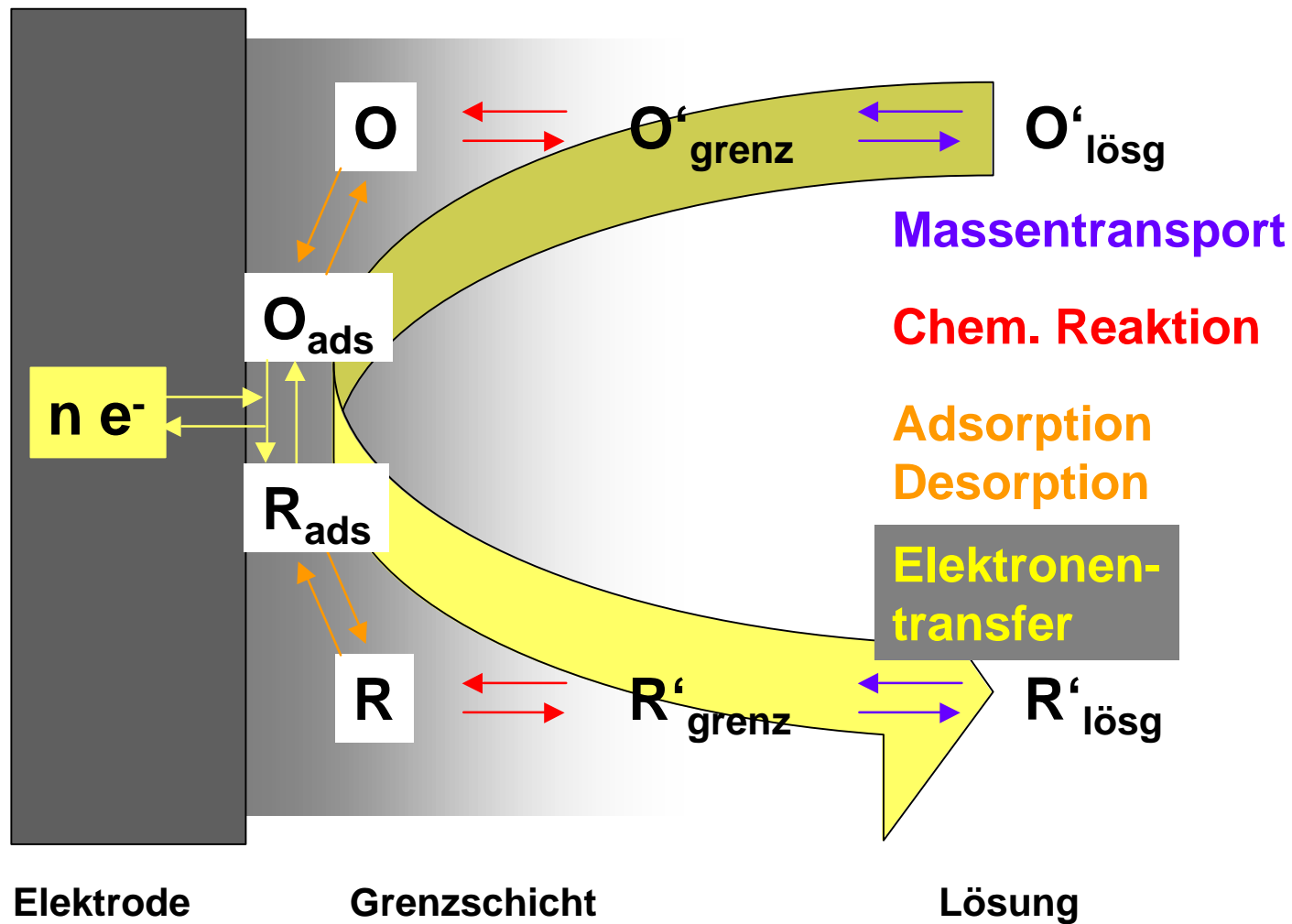
Zyklische Voltammetrie



Mikroelektroden zeigen 2-dim. Diffusion

Redoxreaktion an Elektrodenoberfläche

Teilschritte



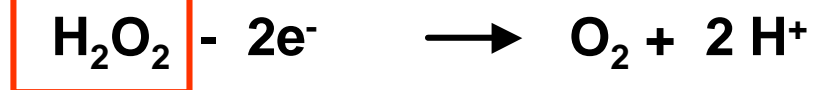
Meßprinzip

Amperometrischer enzymatischer Glucosesensor

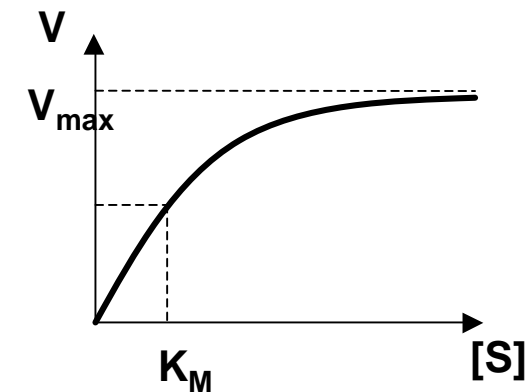
1. Glucose-Oxidase GOD (K_M 33 μ M) - vgl. Glc im Blut ca. 5,5 mM



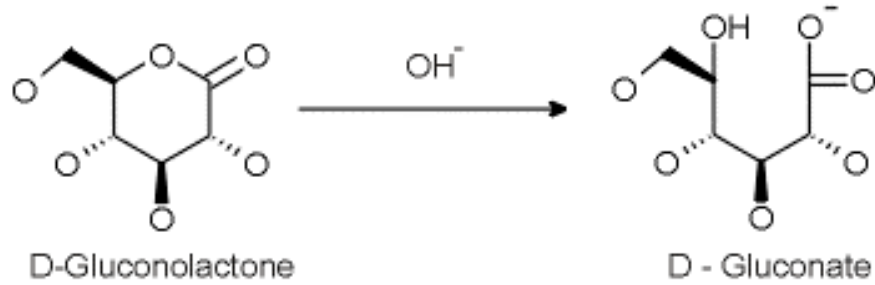
2. Edelmetall-Elektrode bei +0,6 V gegen Ag/AgCl



Andere Analyten: Lactat,

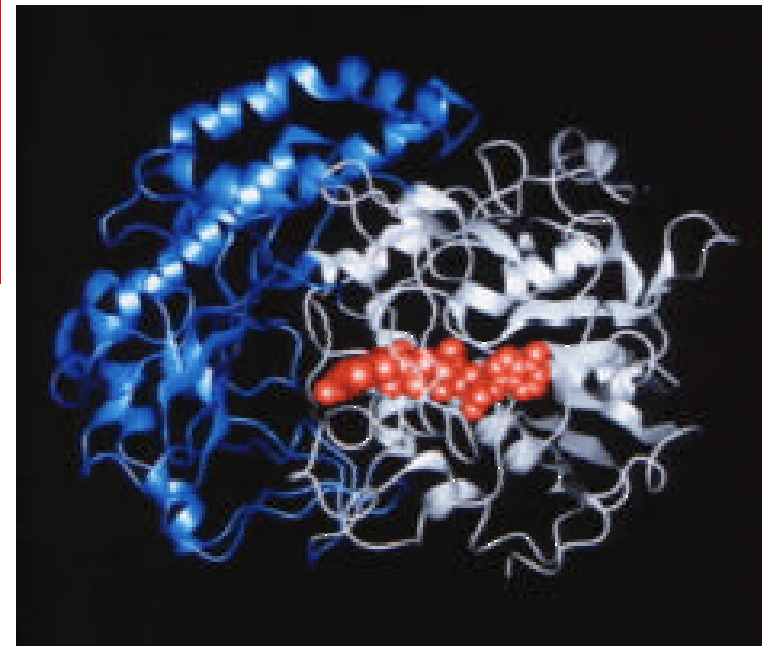


Glucose-Oxidase (E.C. 1.1.3.4)

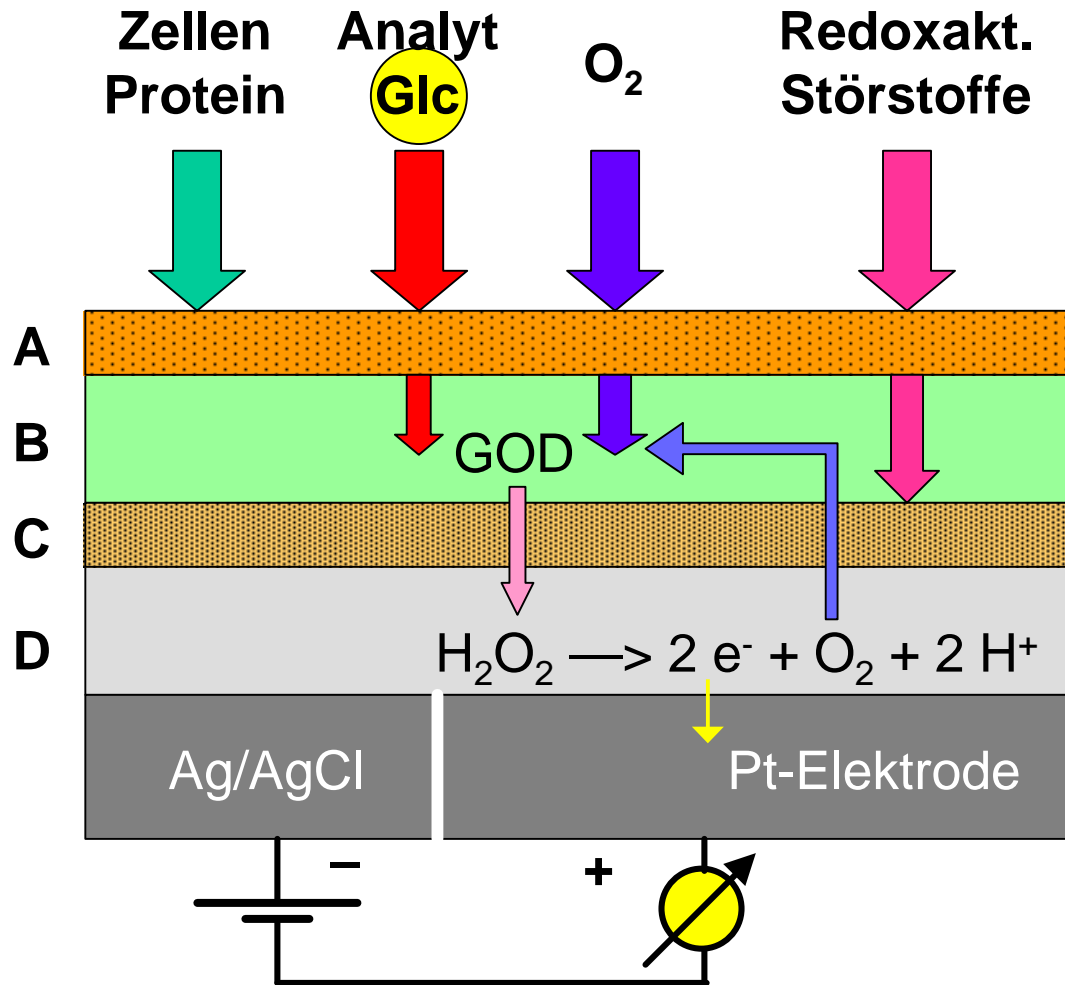


K_M 33 μM

MW 160 kDa



Schematischer Aufbau einer amperometrischen Enzymelektrode (Glucoseoxidase)



Schichtaufbau

A. Äußere Membran

schirmt störende Makromoleküle und Zellen ab, mit Nanoporen, Diffusionsbarriere

B. Enzymschicht

immob. GOD, Reaktionsraum $Glc + O_2 \rightarrow H_2O_2 + \dots$

C. Innere Membran

schirmt redoxaktive niedermolekulare Störstoffe ab

D. Elektrodenraum

Amperometrischer Nachweis von H_2O_2 an Pt-Anode (+ 0,6V gegen Ag/AgCl)
Elektrolytlösung (KCl)

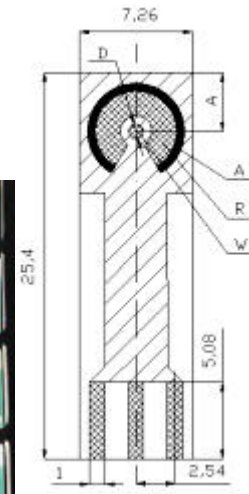
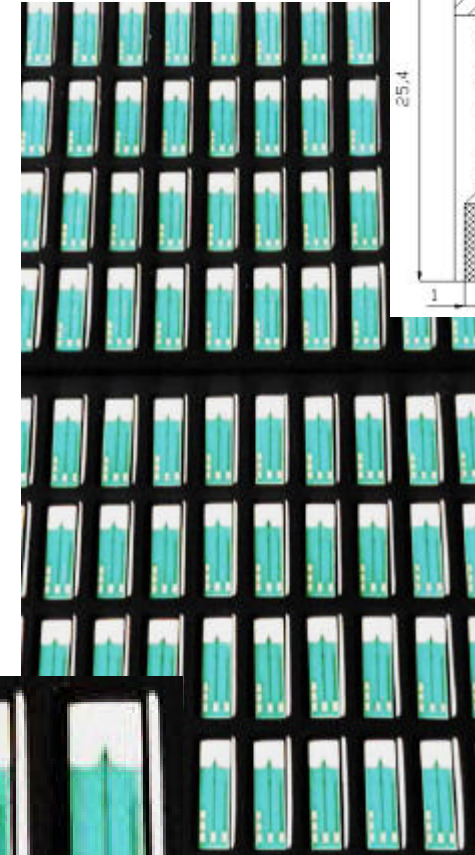
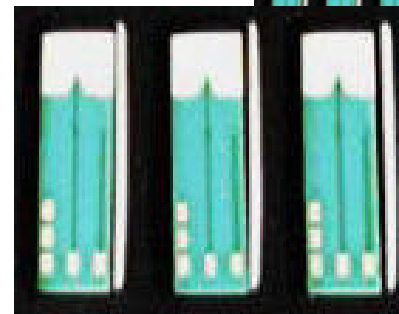
Kommerzieller Glucosesensor

BST Biosensor Technologie GmbH Berlin



Membranen und konv. Elektroden

Dickfilmelektroden
Enzymbeschichtung



www.bst-biosensor.de/

Biosensor in Blutzuckermonitor

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ACCU-CHEK®

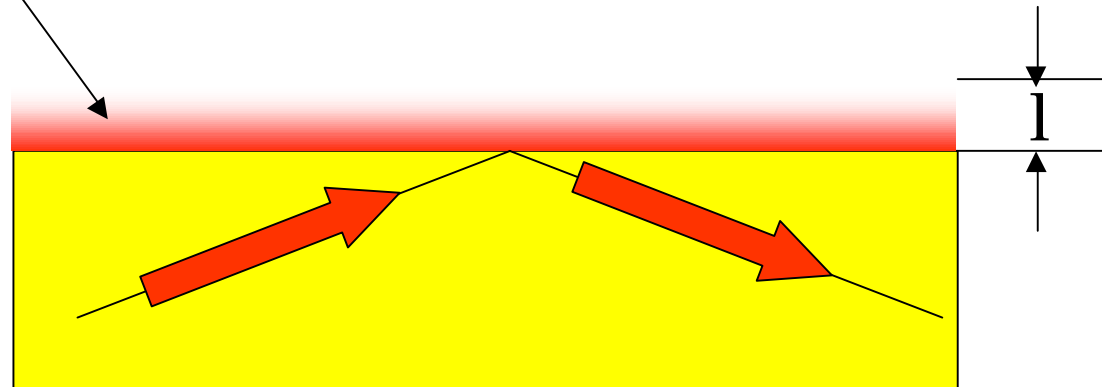
Leben. So wie ich es will.



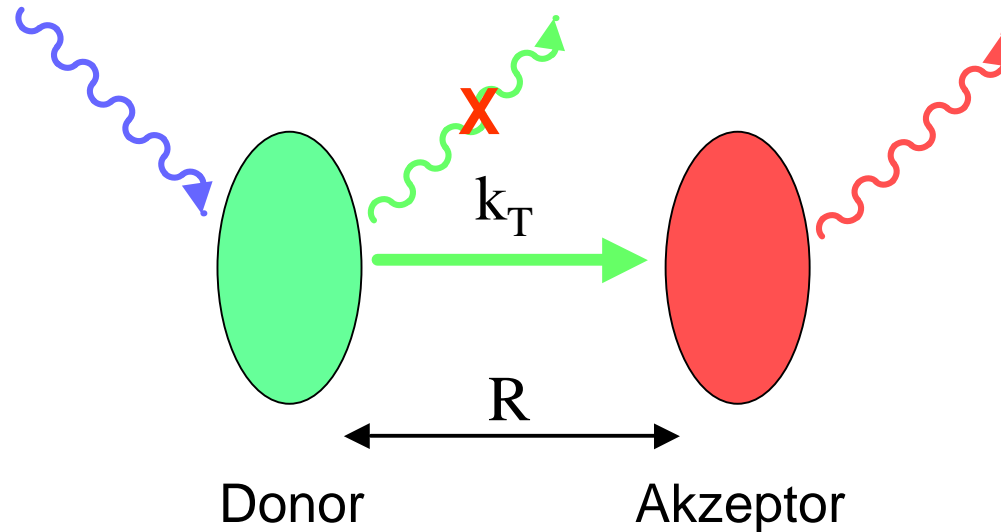
Optische Detektion

- siehe Vorlesungen 10 – 12 -

- Meßparameter
 - Brechungsindex
 - Absorption
 - Emission (Fluoreszenz, Lumineszenz)
- Spez. Grundlagen
 - Evaneszente Welle (bei Totalreflektion; z. B. in Lichtleitern, opt. Fasern)



Fluoreszenz-Resonanz-Energie-Transfer FRET



X Emission des Donors verschwindet

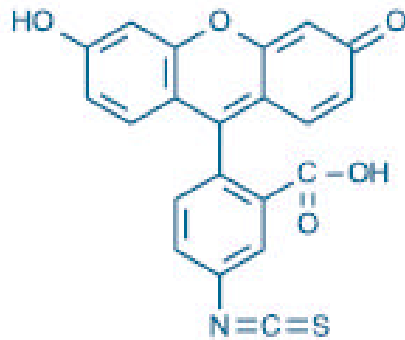


Strahlungslose Energieübertragung
Förster-Mechanismus (Dipol-Resonanz)
Transferrate

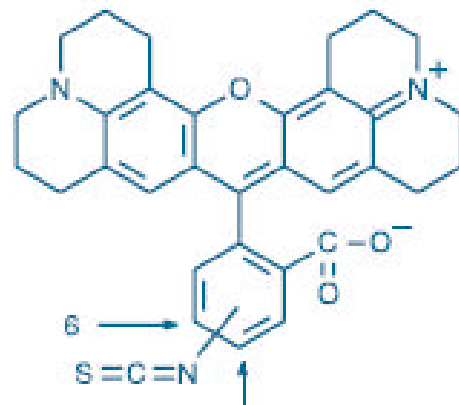
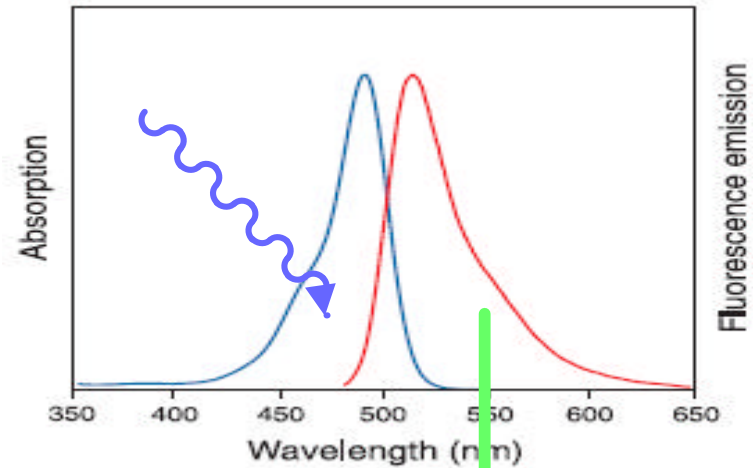
$$k_T = \frac{1}{\tau_d} \left(\frac{R_0}{R} \right)^6$$

τ_d F-Lebensdauer des Donors, $R_0 \sim 25 - 55 \text{ \AA}$

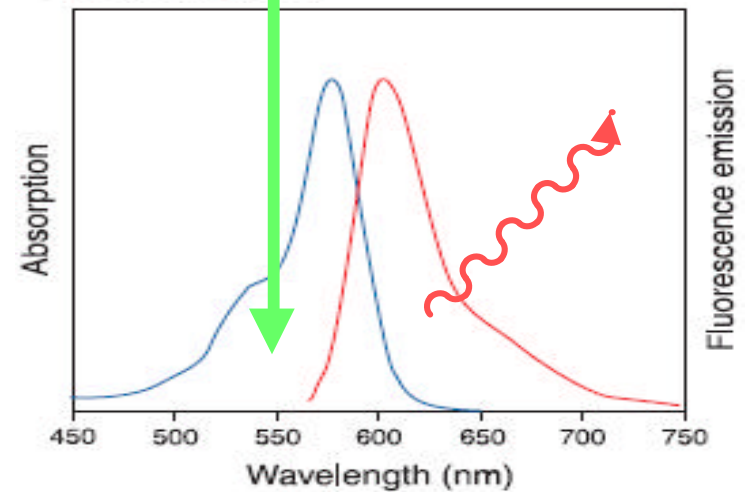
FRET - Fluoreszein/Rhodamin als Donor/Akzeptor



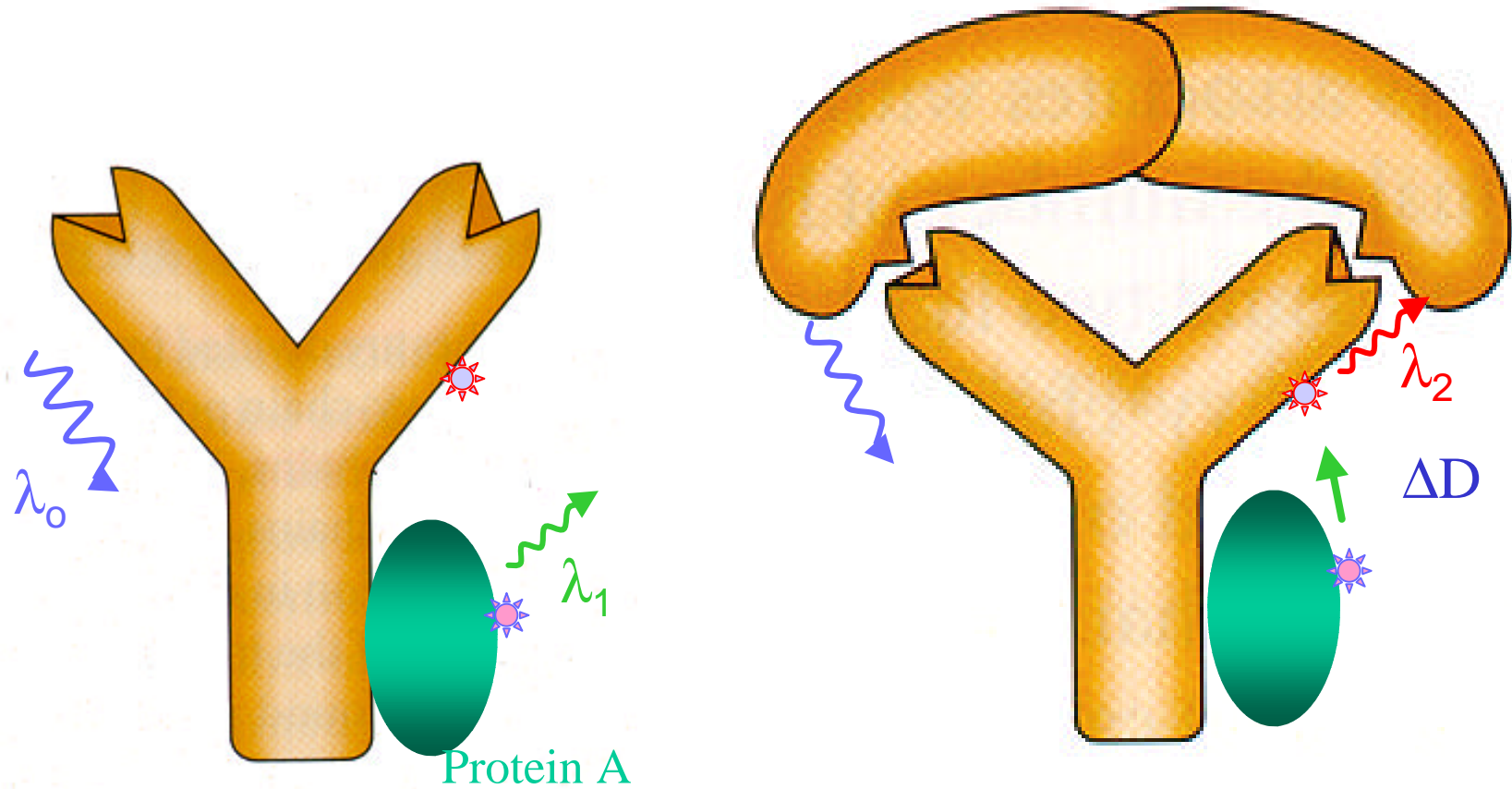
Fluoreszein



Rhodamine



FRET Immunosensor “molecular beacon”



S.A. Grant et al.

GENETIC ENGINEERING NEWS

Volume 23, Number 11
June 1, 2003

dates
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GEN

NEWS

INTERNATION

Microarrays Are on the Rise

Old and New Uses Drive Renewed Interest in Oligonucleotide Arrays

Nina Flanagan

The future of microarray technology points to new applications and emerging trends, as made clear at the recent Cambridge HealthTech Institute Conference "Macroresults for Microarrays" held in Boston. Overall, there is a big push to enhance the quality of microarrays, to discover new applications, such as toxicology, and to provide microarrays of new species.

Sunny W. Tam, Ph.D., former associate director of cell biology at **Encysive Pharmaceuticals** (formerly Texas Biotechnology; Houston), discussed the applications of microarray technology for drug discovery. Such uses include studies on drug target validation, drug mechanisms of action, and preclinical development.

Dr. Tam and colleagues constructed an antibody microarray to examine animal toxicology. The assay rapidly ana-



lyzed multiple cytokines that are indicative of toxic response from a small sample. Performance of the array on MCP-1 expression of animal sepsis samples was compared with conventional ELISA and was found to be reproducible and concordant with ELISA results.

The researchers concluded that this assay could be automated to measure expression levels of multiple inflammatory Th1/Th2 cytokines simultaneously from a range of between tens and hundreds of biological samples. "In the drug industry, you

See Microarrays page 19

Integration vieler Biosensoren -> Biochip

Vorteile und Probleme

- **Miniaturisierung**
 - Verminderung von Reagentienverbrauch, Analysendauer
 - Problem der Detektion (Nachweisgrenze) und Genauigkeit
- **Parallelisierung (Array)**
 - Hoher Durchsatz (HTS, high throughput screening > 100.000 Tests/d)
 - Automatisierung, Robotik
 - Multi-Analyt Detektion
 - Problem der reproduzierbaren Probenvorbehandlung
- **Integrationsdichte**
 - Herstellungstechnologien
 - Kosten/Chip - Kosten/Analyse, Analysenzahl

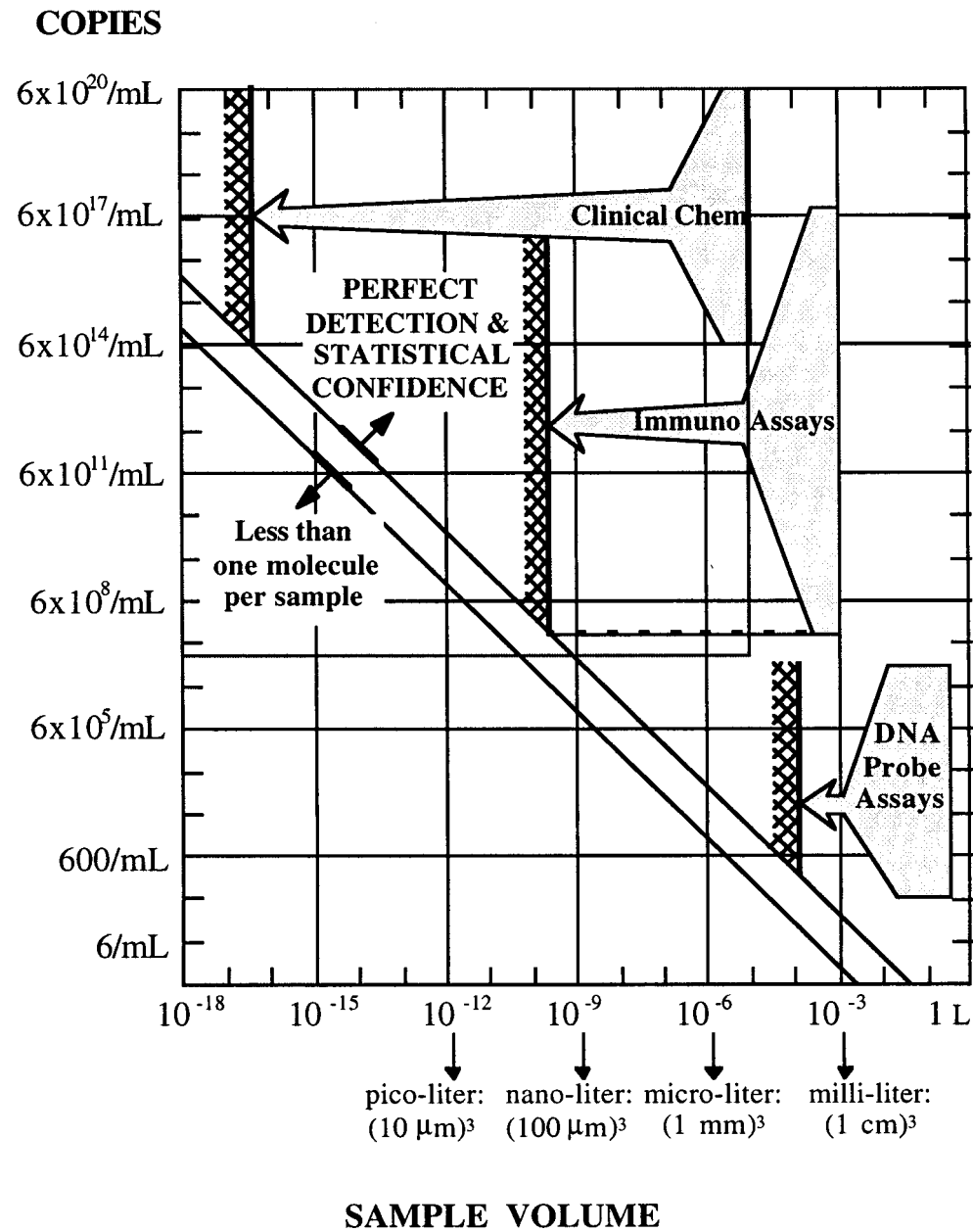
Typische Analyten in der Diagnostik

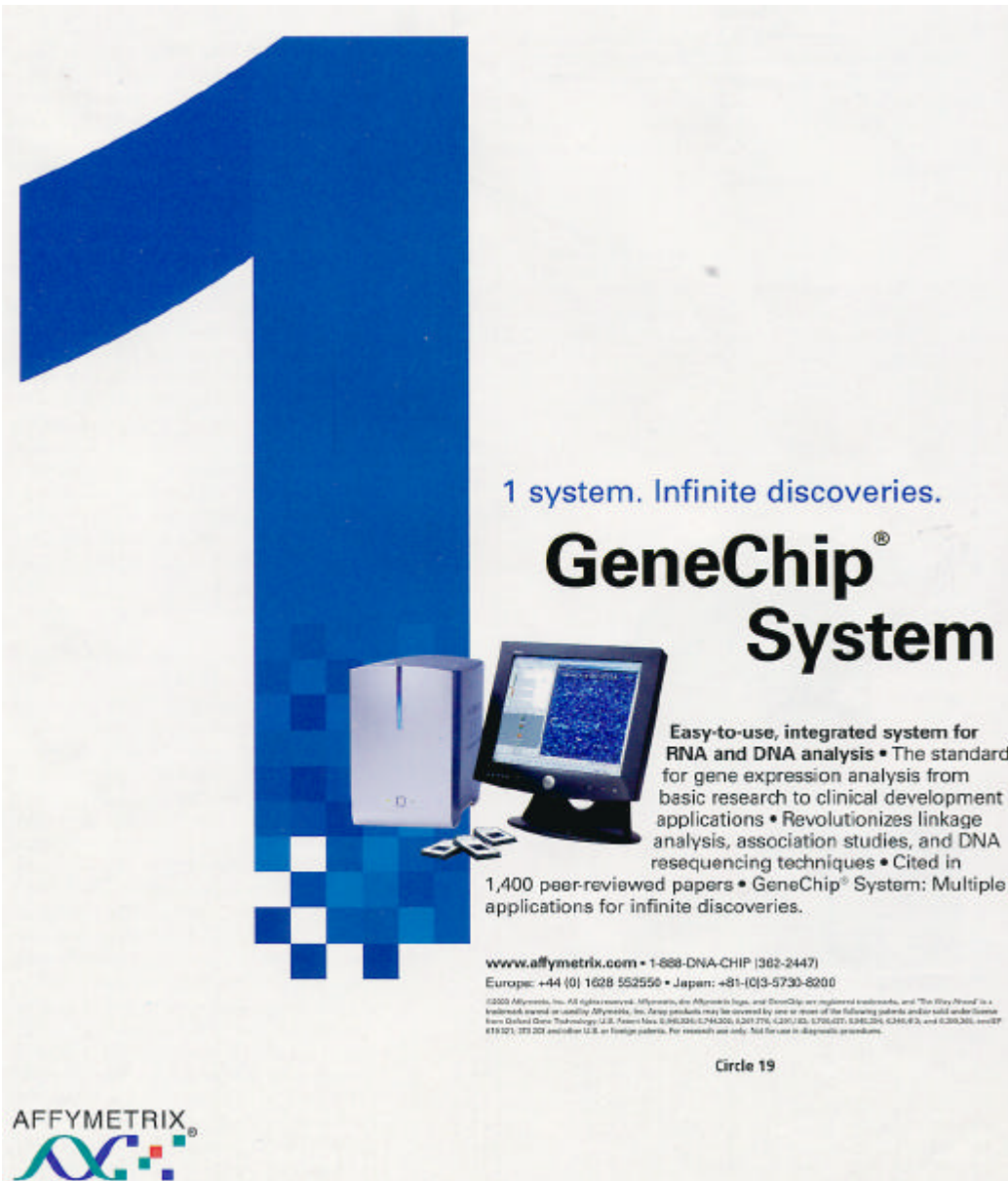
Table I. Concentrations of typical diagnostic analytes in human blood or other samples

Biochemical analyte	Typical molecules or copies/mL
Sodium	3×10^{19}
Glucose	1×10^{18}
Cholesterol	8×10^{17}
Uric Acid	8×10^{16}
Creatinine	3×10^{16}
Theophylline	3×10^{16}
Carbamazepine	1×10^{16}
Cortisol	3×10^{13}
Digoxin	8×10^{11}
Specific Abs	—
Estrogens	4×10^{10}
DNA finger-printing and genetic disorder pre-disposition	$10^6 - 10^7$
Cancer detection and analysis	$\ll 10^4$
HIV in blood (asymptomatic)	< 400
Gm- bacteremia (whole blood)	< 10
EHEC in carcass washings	< 1
Biothreat agents in air (after concentration)	< 100
Cryptosporidium in drinking water	$< 2 \times 10^4$
Food poisoning agents (e.g., <i>Salmonella sp.</i> , <i>E. coli</i> O157:H7, <i>Campylobacter jejuni</i> , <i>Vibrio cholerae</i> , <i>Listeria monocytogenes</i> , <i>Shigella sp.</i> , <i>Bacillus cereus</i> , <i>Yersinia enterocolitica</i> , <i>Clostridium perfringens</i>)	< 1

Miniaturisierung und Nachweisgrenze

Limitierung der Probe
- durch Menge
- durch Konzentration





1 system. Infinite discoveries.

GeneChip® System

Easy-to-use, integrated system for RNA and DNA analysis • The standard for gene expression analysis from basic research to clinical development applications • Revolutionizes linkage analysis, association studies, and DNA resequencing techniques • Cited in 1,400 peer-reviewed papers • GeneChip® System: Multiple applications for infinite discoveries.

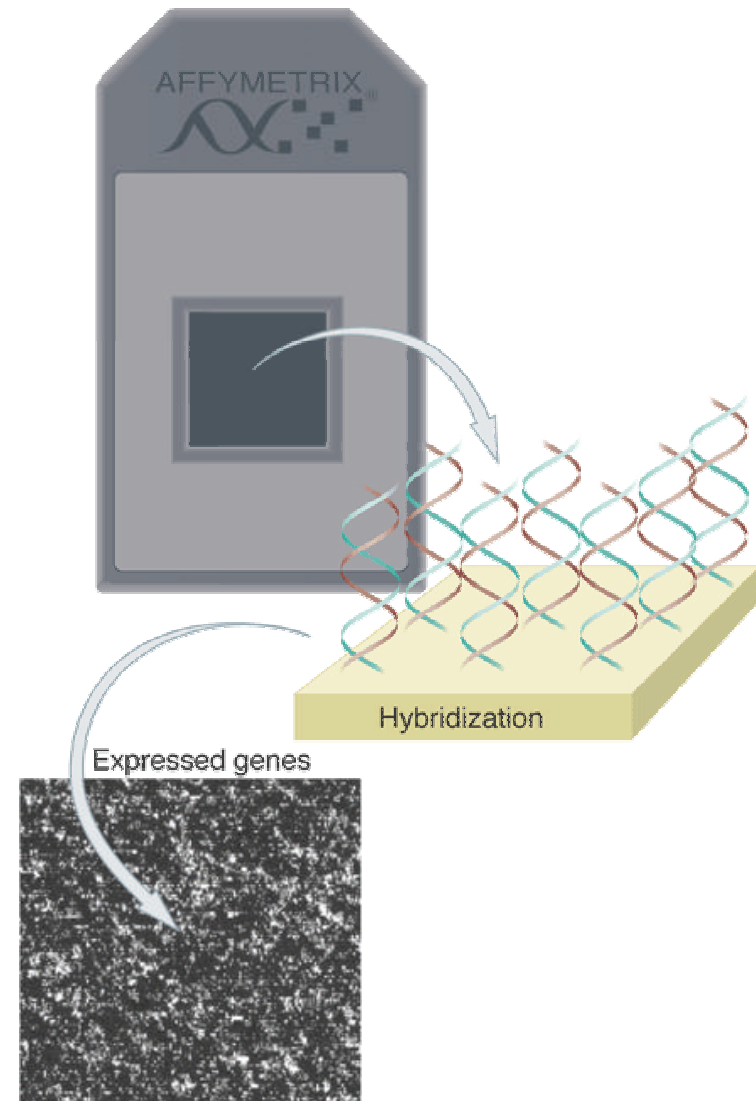
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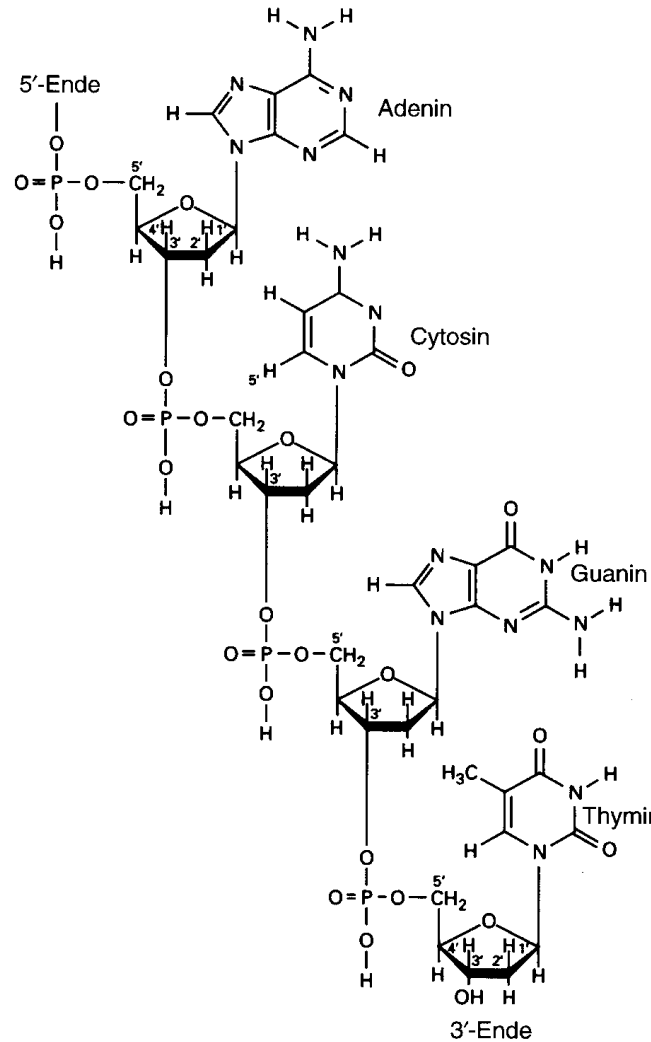
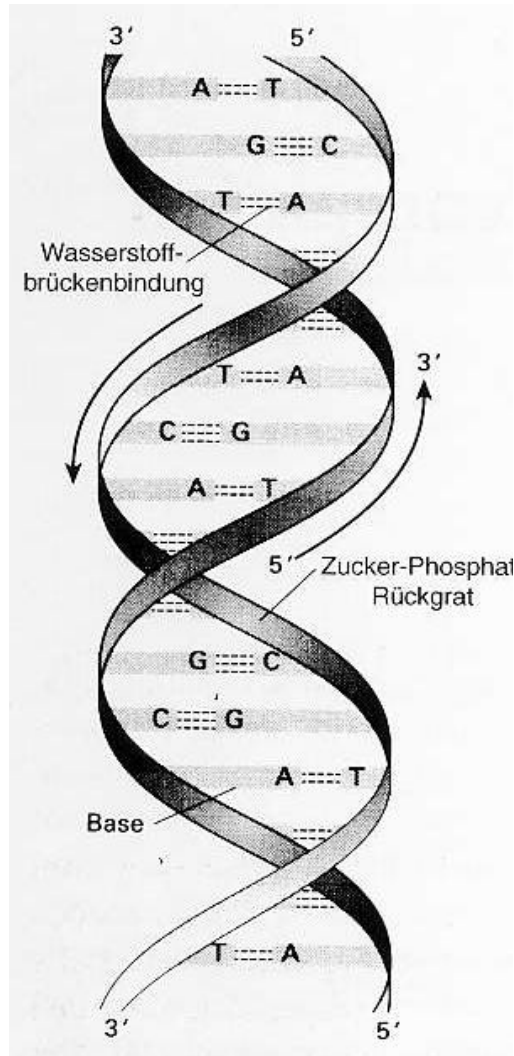
Circle 19

AFFYMETRIX®

DNA Microarrays



DNA-Struktur und Hybridisierung



Hybridisierung

abhängig von

- Temperatur
 - Salzkonzentration
- “Stringenz”

Ziel

Detektion von
1 Mismatch
in 20 - 60 Bp

Aufgaben von Oligonukleotid-Arrays

Aufbau und Zweck

- Auf Träger (Glas) gebundene Sonden: DNA, Oligos von 20 - 60 Basen
- Nachweis der komplementären Sequenz durch Hybridisierung

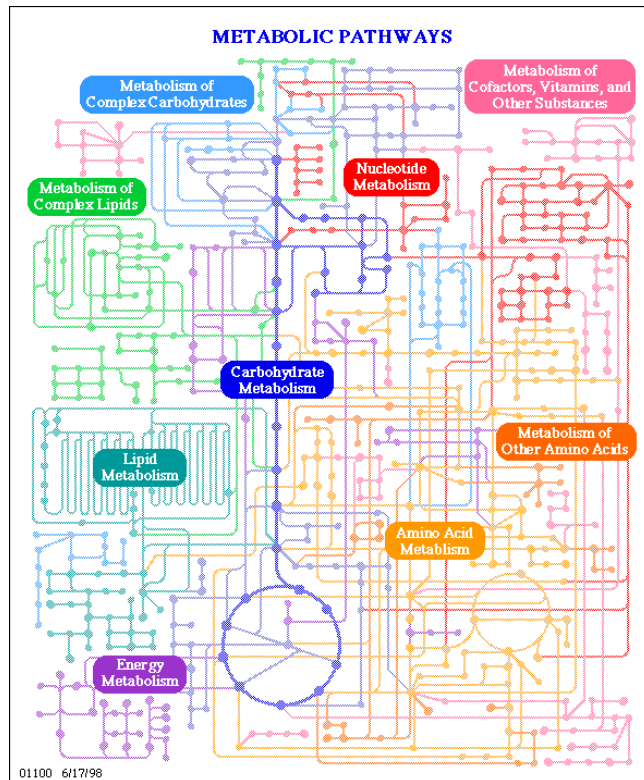
Anwendungen

- Sequenzierung durch Hybridisierung (SBH), redundante und überlappende Sonden decken die Sequenz ab
- Expressionsanalyse, Quantifizierung von Messenger-RNA als Maß für Proteinsynthese
- Detektion von Mutanten des Genoms (single nucleotide polymorphism, SNP) – “SNP-Detektion”
- Detektion von DNA von infizierenden Bakterien oder Viren “Infektionsdiagnostik”

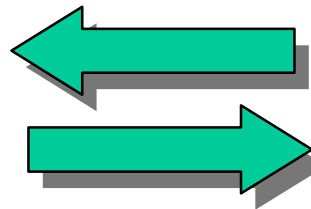
DNA-Chips zeigen die Genexpression

Die Genexpression beschreibt die Bildung einzelner Proteine aus dem Genom (Bauplan)

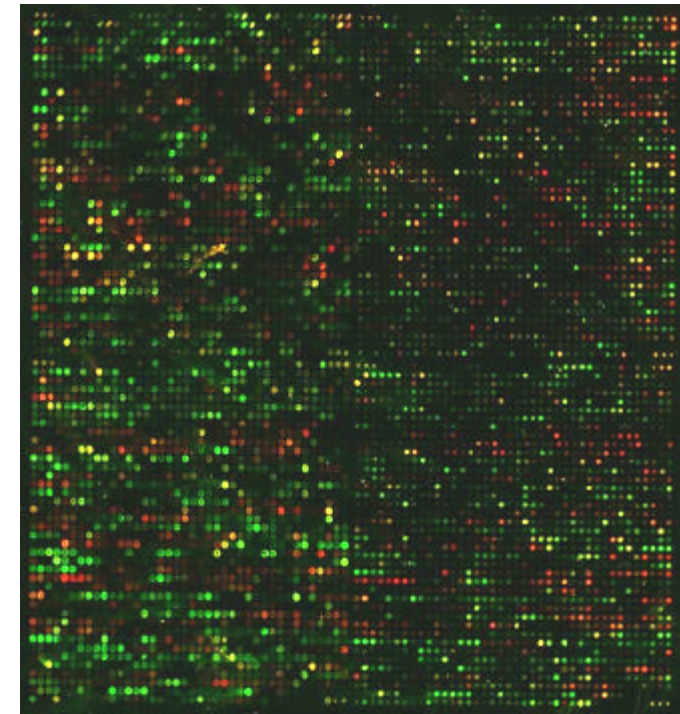
Genom: Auf dem DNA-Chip (Originalgröße: 18mm x 18mm) sind 6116 Hefegene repräsentiert



Welche Gene sind wann aktiv?



Welche Gene gehören zusammen?

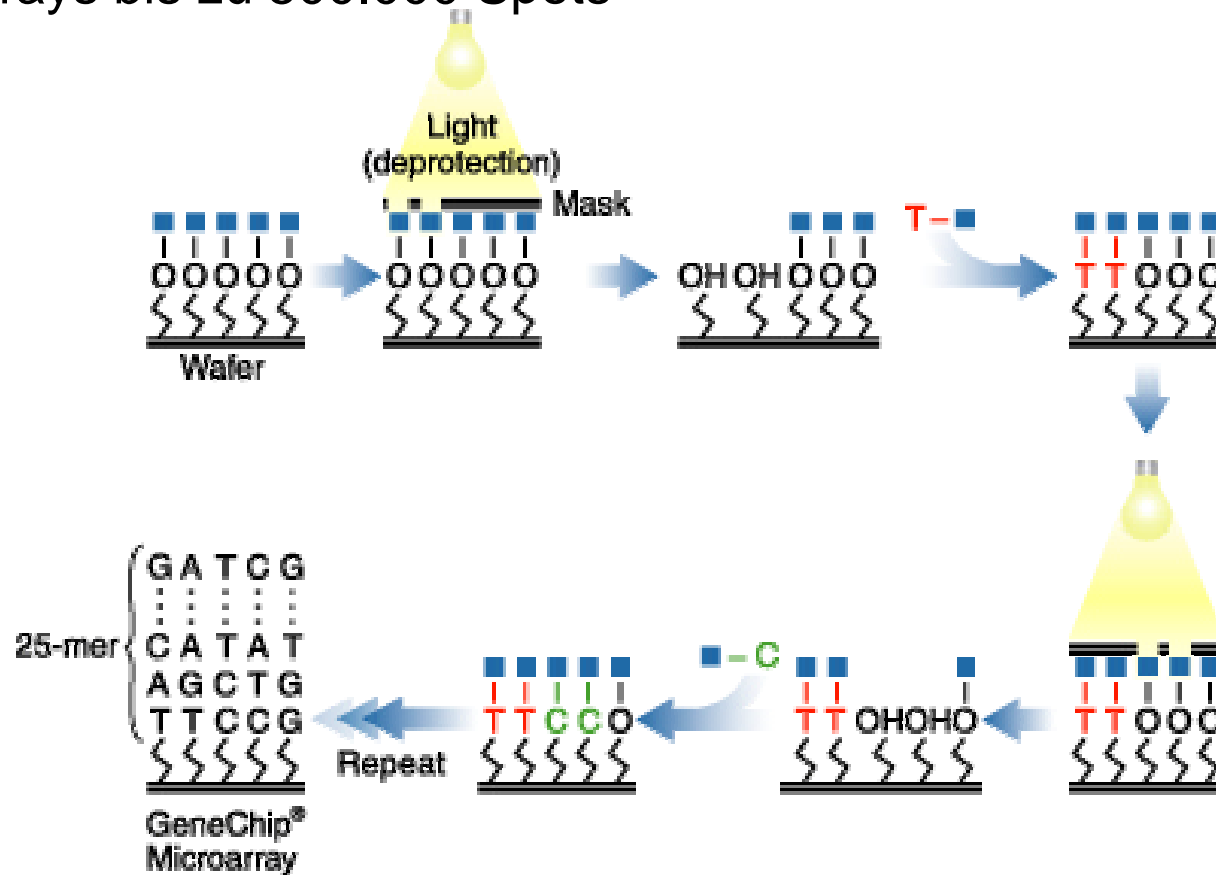


Proteom: Stoffwechselkarte

Grün: Gene, die bei hohem Glucosegehalt stärker exprimiert werden.
Rot: Gene, die bei Glucosemangel verstärkt exprimiert werden.
Gelb: Gene, deren Expression unverändert bleibt.

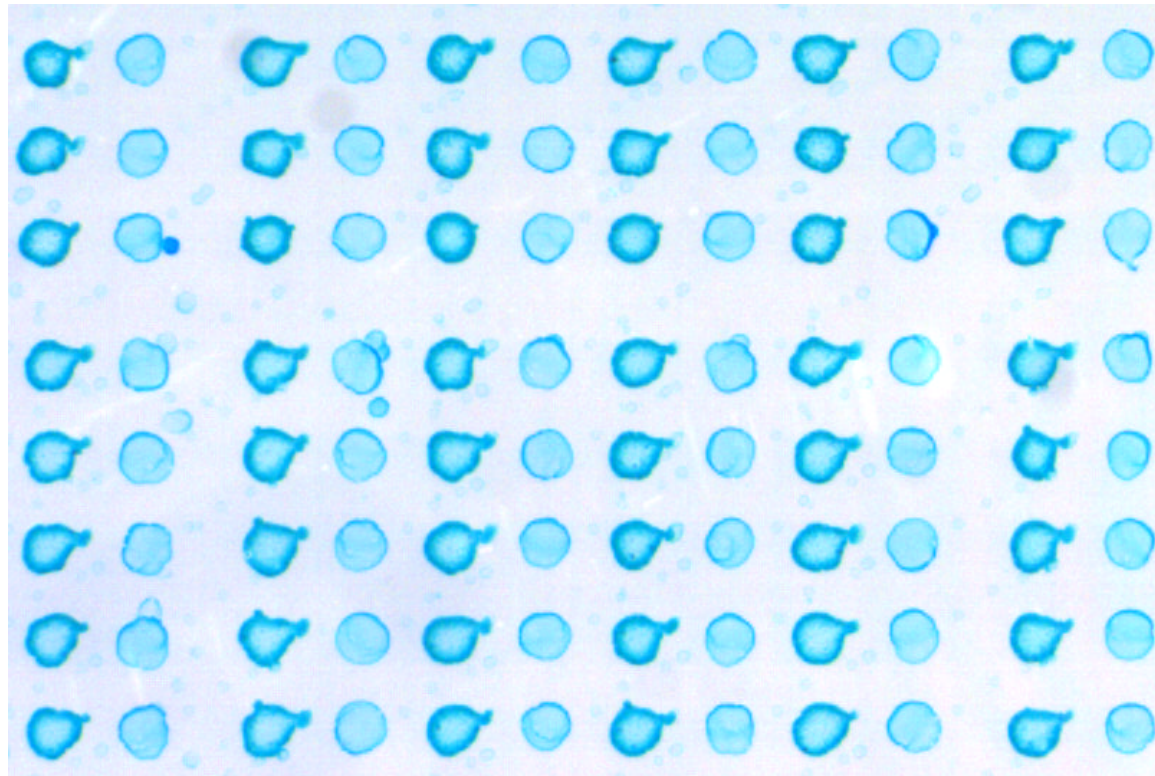
Herstellung von Oligonukleotid-Arrays 1

- Licht-gesteuerte Synthese (Photolithographie), Licht entfernt eine Schutzgruppe - Frage: Signal/Rauschen?
- Arrays bis zu 500.000 Spots



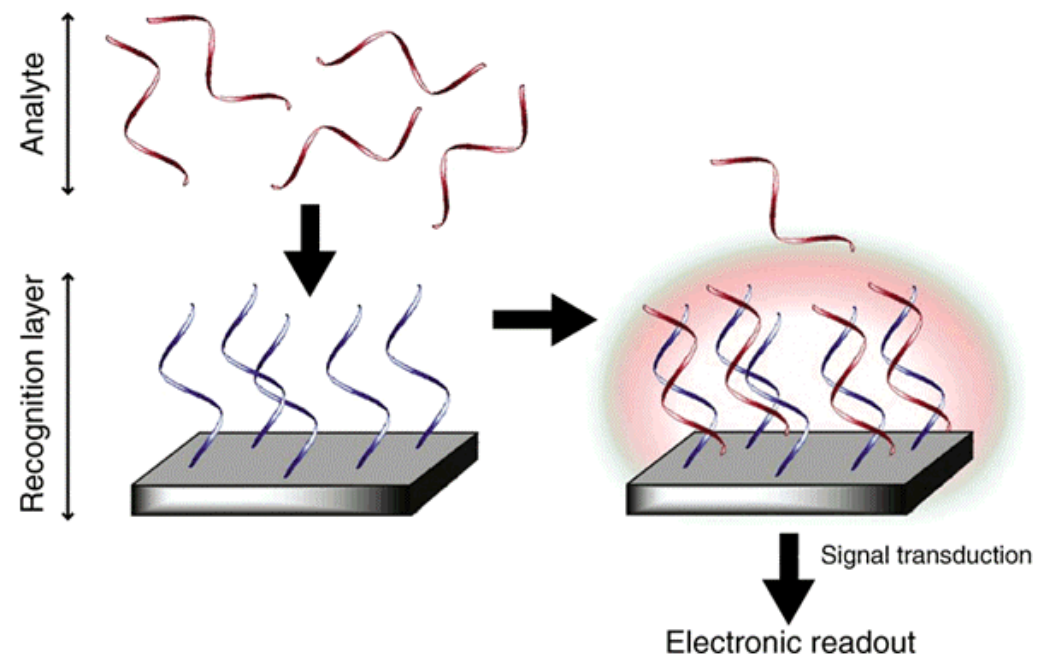
Herstellung von Oligonukleotid-Arrays 2

- Aufbringen von Mikrotropfen (microspotting, microprinting, needle array) (vgl. Tintenstrahldrucker)
- Technisches Problem der individuellen Dosierung

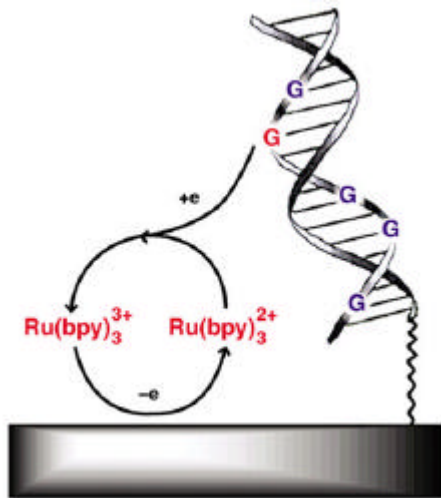


Detektion der Hybridisierung

- Fluoreszenz
 - Einbau einer Fluoreszenzmarkierung
 - molecular beacon (FRET)
- Elektrochemische Detektion

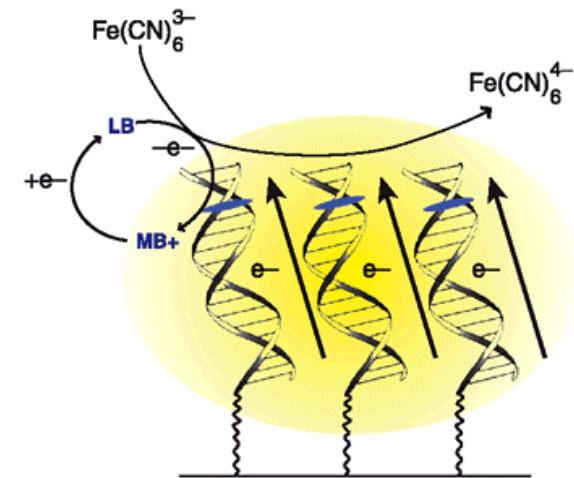
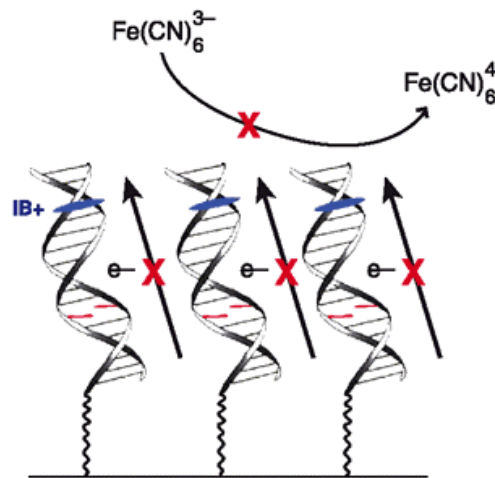


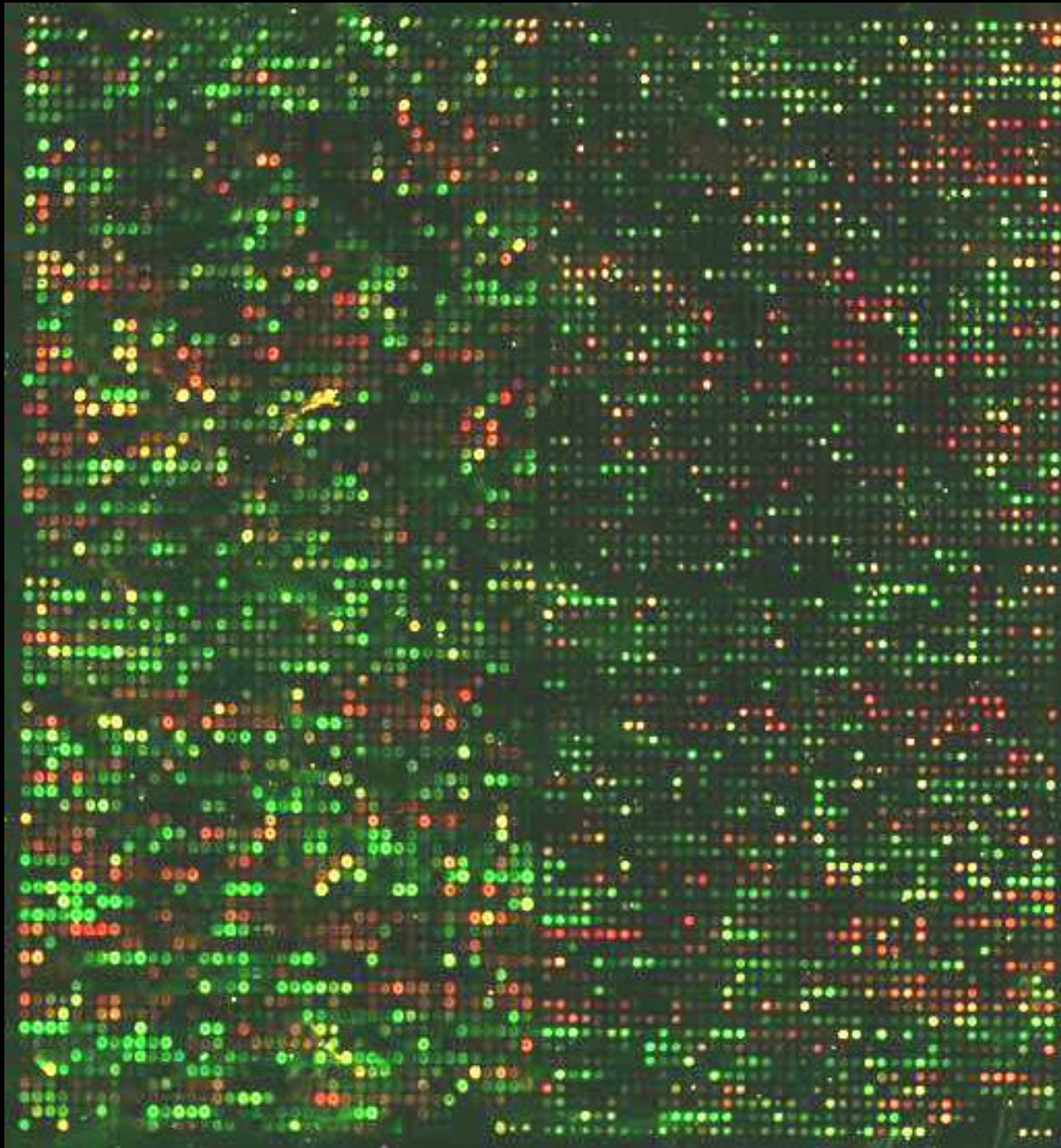
Elektrochemische DNA-Chips (2 Beispiele)



1. Mediator bewirkt Elektronentransfer von Poly-G zu Elektrode -> Strom

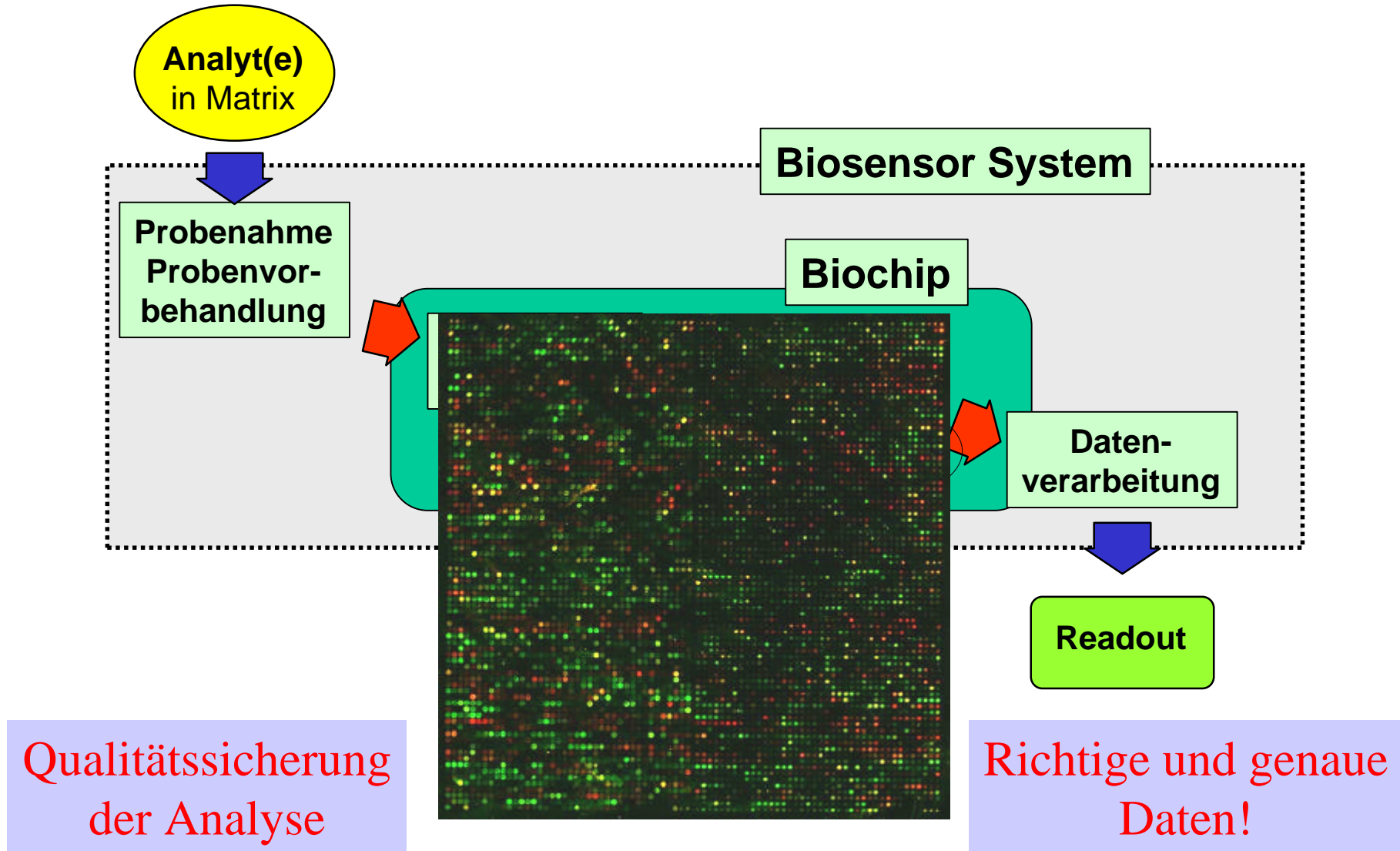
2. Mismatch unterbricht Pfad für Elektronentransfer zur Elektrode -> Strom bei match



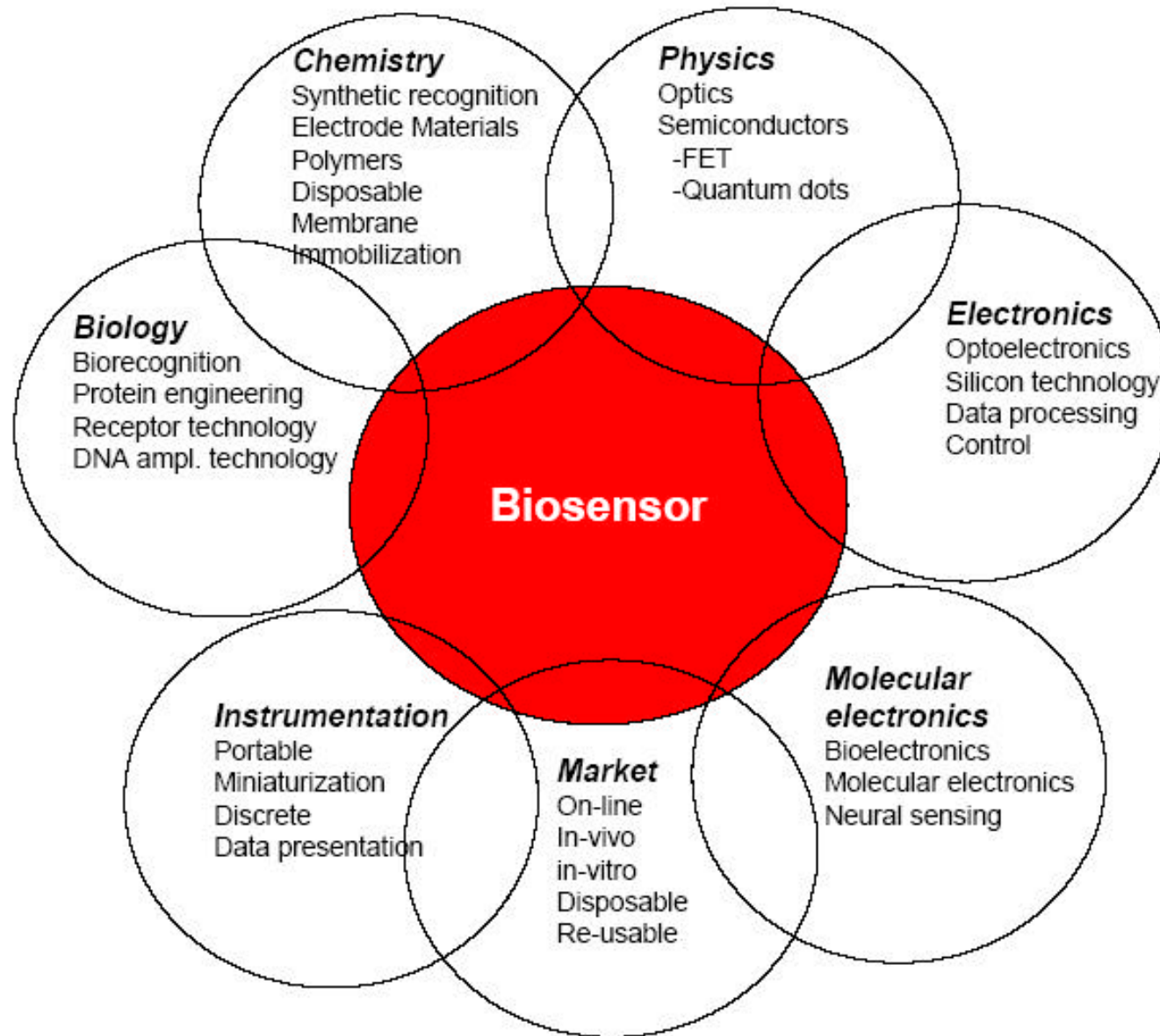


Fluoreszenz-
detektion

Biochip und Biosensor - Meßunsicherheit und Datenqualität („GIGO“)



Biosensoren und Biochips beruhen auf vielen Disziplinen!



chem. Signal



elektr. Signal



Vielen Dank!