Wahlpflichtfach „Vertiefung Physikalische Chemie“
Masterstudiengang Modul WP04

Moderne Methoden der Spektroskopie

Prinzip Microarrays und Biosensoren

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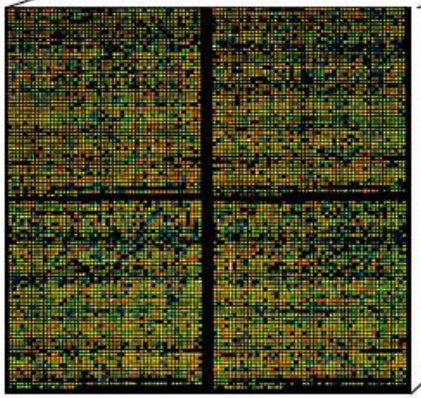
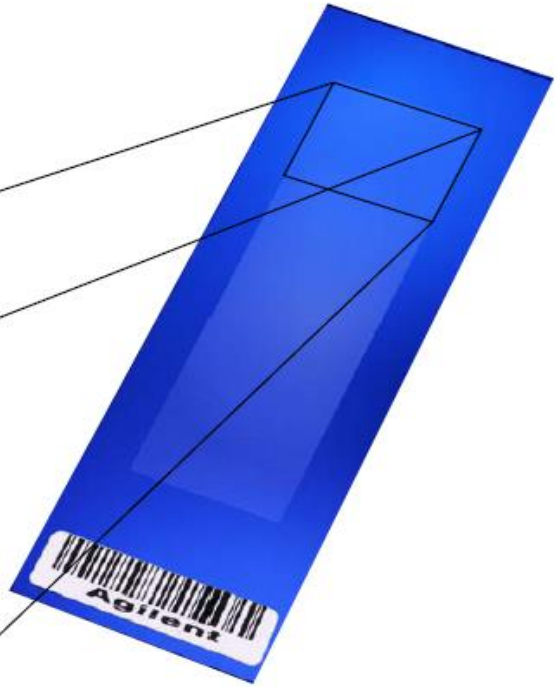
What is a Microarray?

A microarray is a spatially ordered, minituarized arrangement of a multitude of immobilized reagents

First publication:
Roger Ekins et. al.,
„Multi-analyte immunoassay“
1989, J. Pharm. Biomed.
Anal. 7: 155 - 168





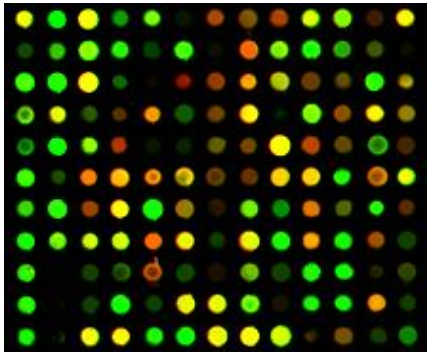
Why DNA Microarrays?

- Genomics → 40-80000 Genes
- Gene expression (Mutation, Polymorphism) of single cell populations

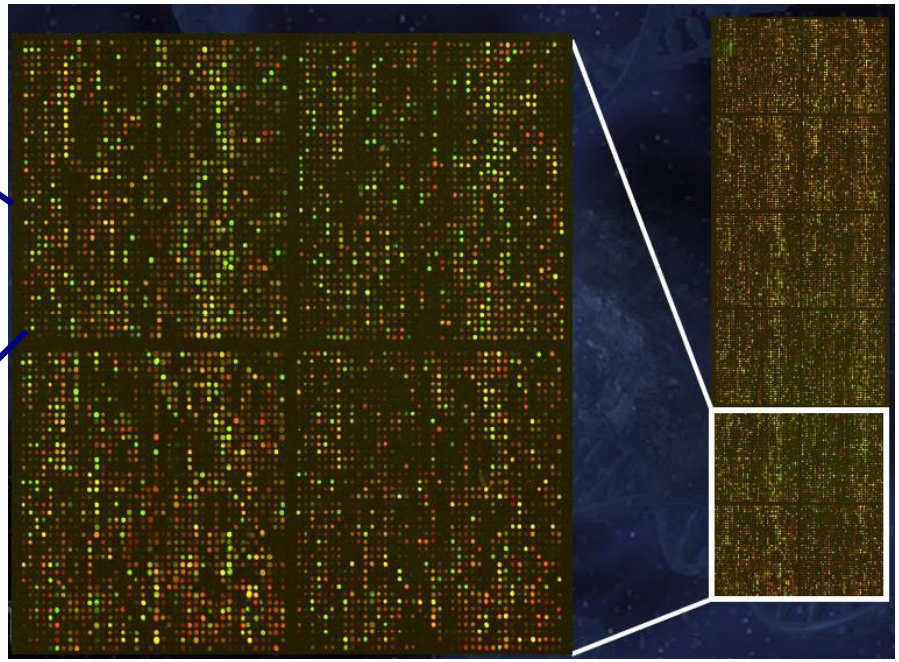
- Miniaturization → simultaneous analysis
  → High Throughput Analysis ⇒ Microarrays

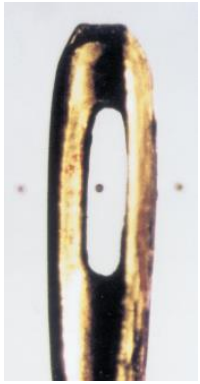
- Nylon Membranes 80-100 spots/cm^2
- DNA Chips up to 10000 spots/cm^2

  - Size of a Spot > 500 μm → Macroarray, < 500 μm → Microarrays

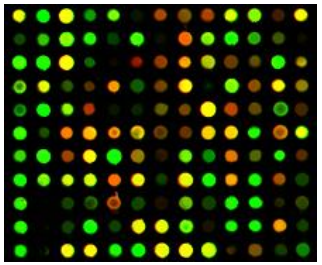


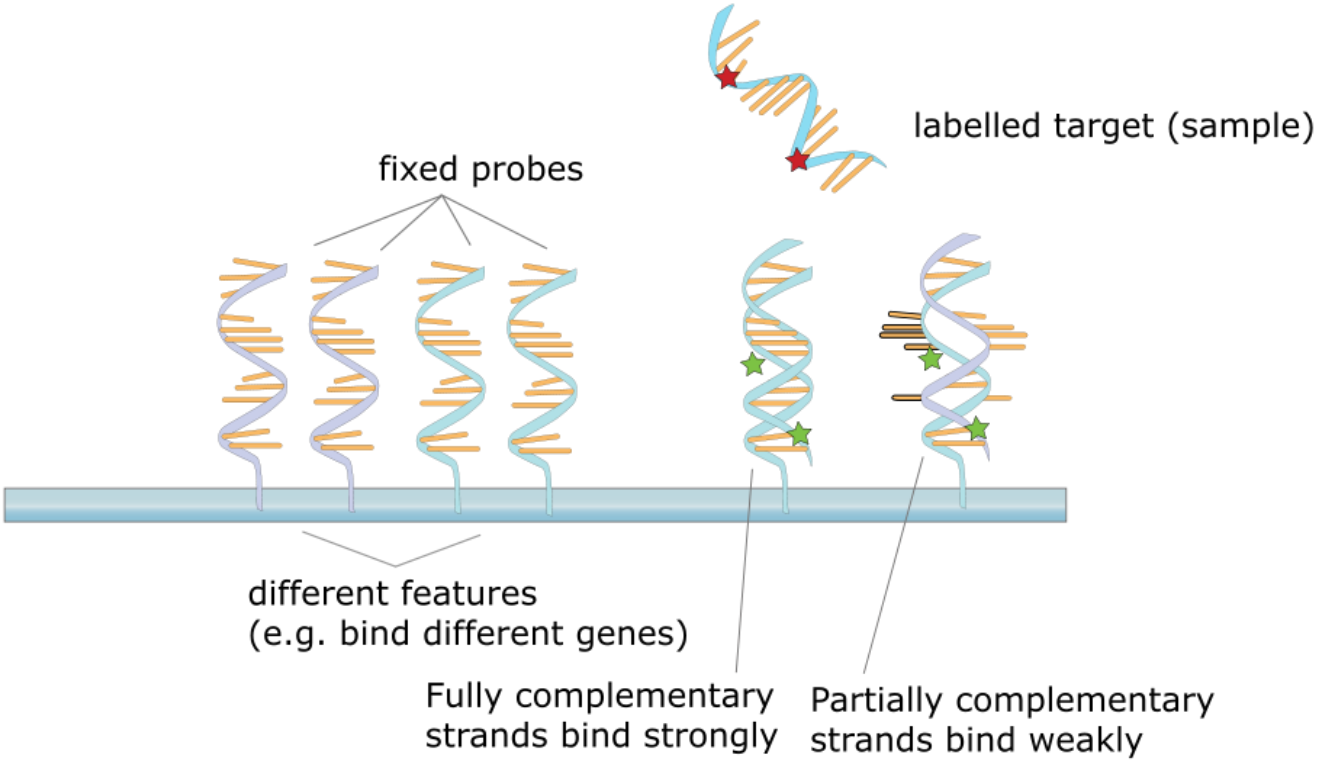
Spot: 50 – 200 μm





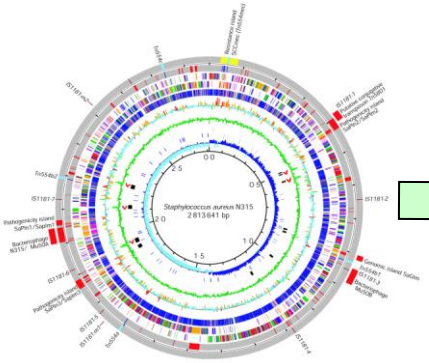
The Principle of DNA Microarrays



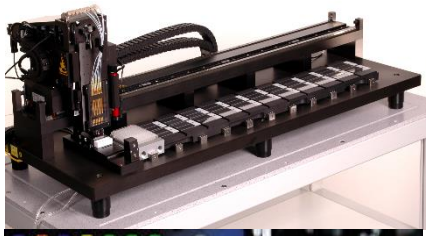


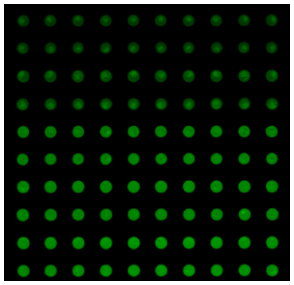
DNA-Microarrays → Workflow

Bioinformatic →Probe Design+ Synthesis

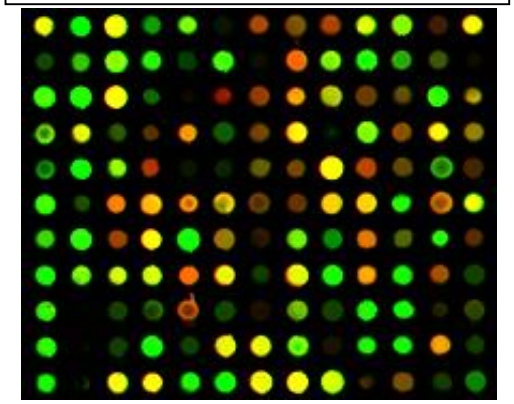


Array Printing+ Immobilization

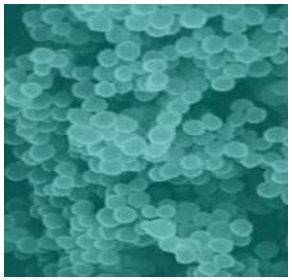


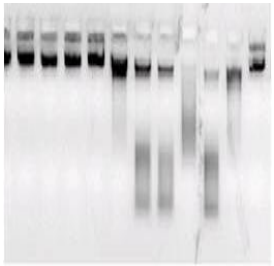


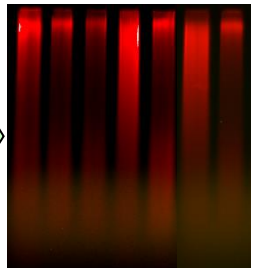
Hybridization,Scannen



Sample preparation →Extraction of Nucleic Acids, Amplification/ Labeling of DNA/ RNA



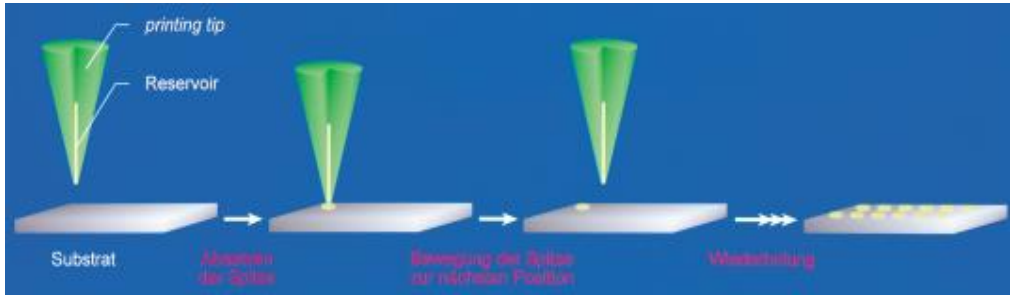




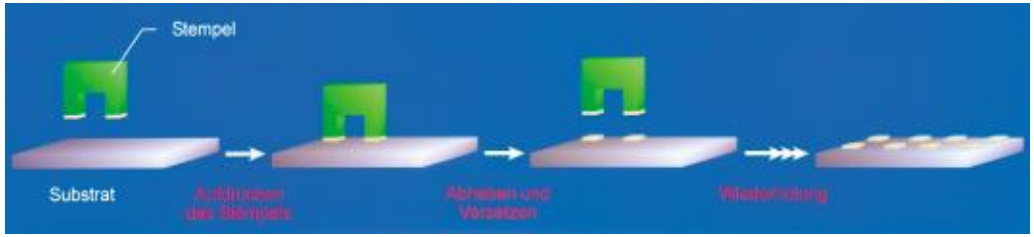
Data Analysis

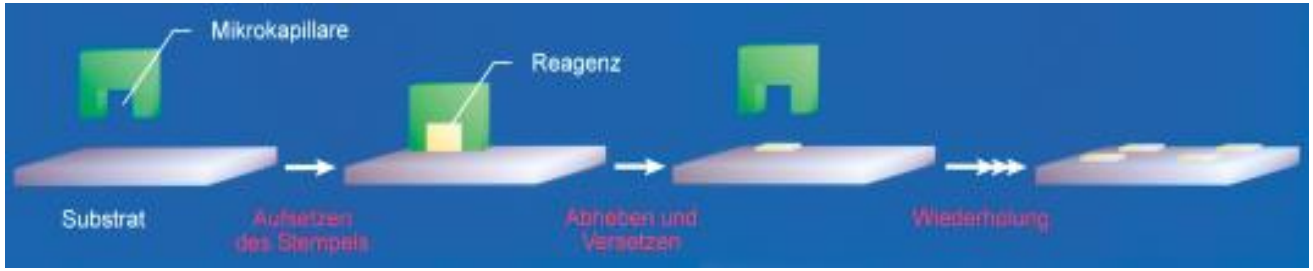
Deposition Techniques (Printing, Spotting)

Contact tip

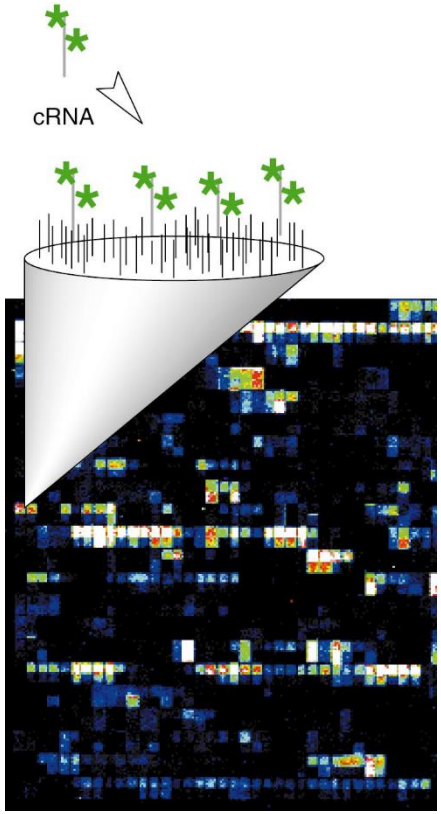


Microcontact printing





Microarrays for Gene Expression Profiling

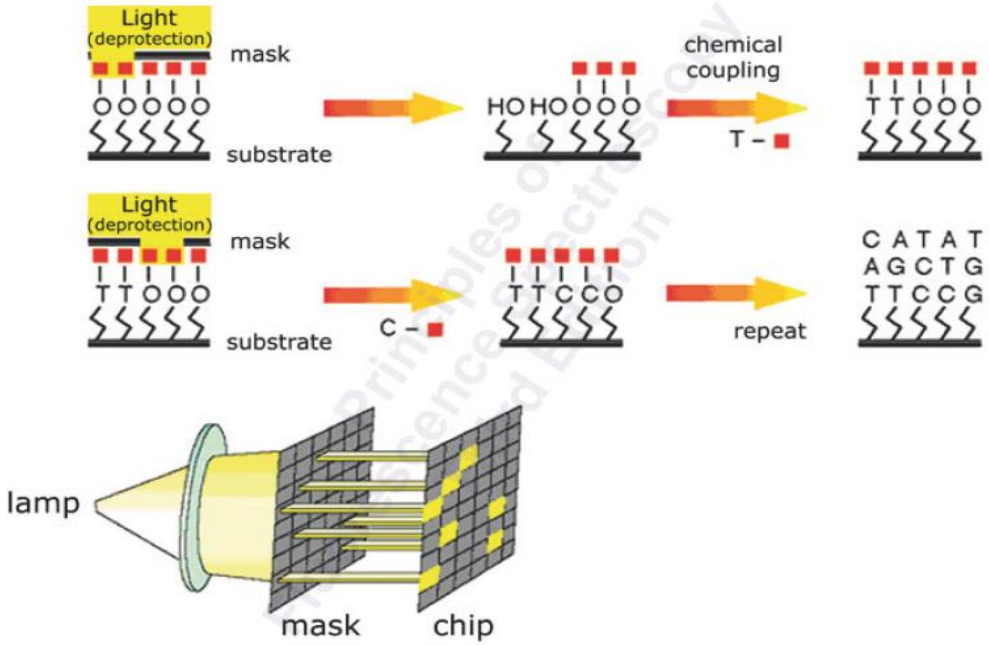


Hybridization of immobilized probe DNA of known sequence with target DNA carrying a fluorescent marker

➔ ***In-situ*** **solid phase synthesis (directly on the chip) of DNA by photolithography**

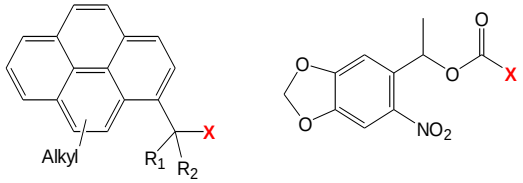
Oligonucleotide Array
→ Affymetrix
D.J. Lockhart et al.
Nat. Biotechn. **1996**, 14, 1675

Photolithographic in-situ Synthesis of Oligonucleotide Arrays



Affymetrix

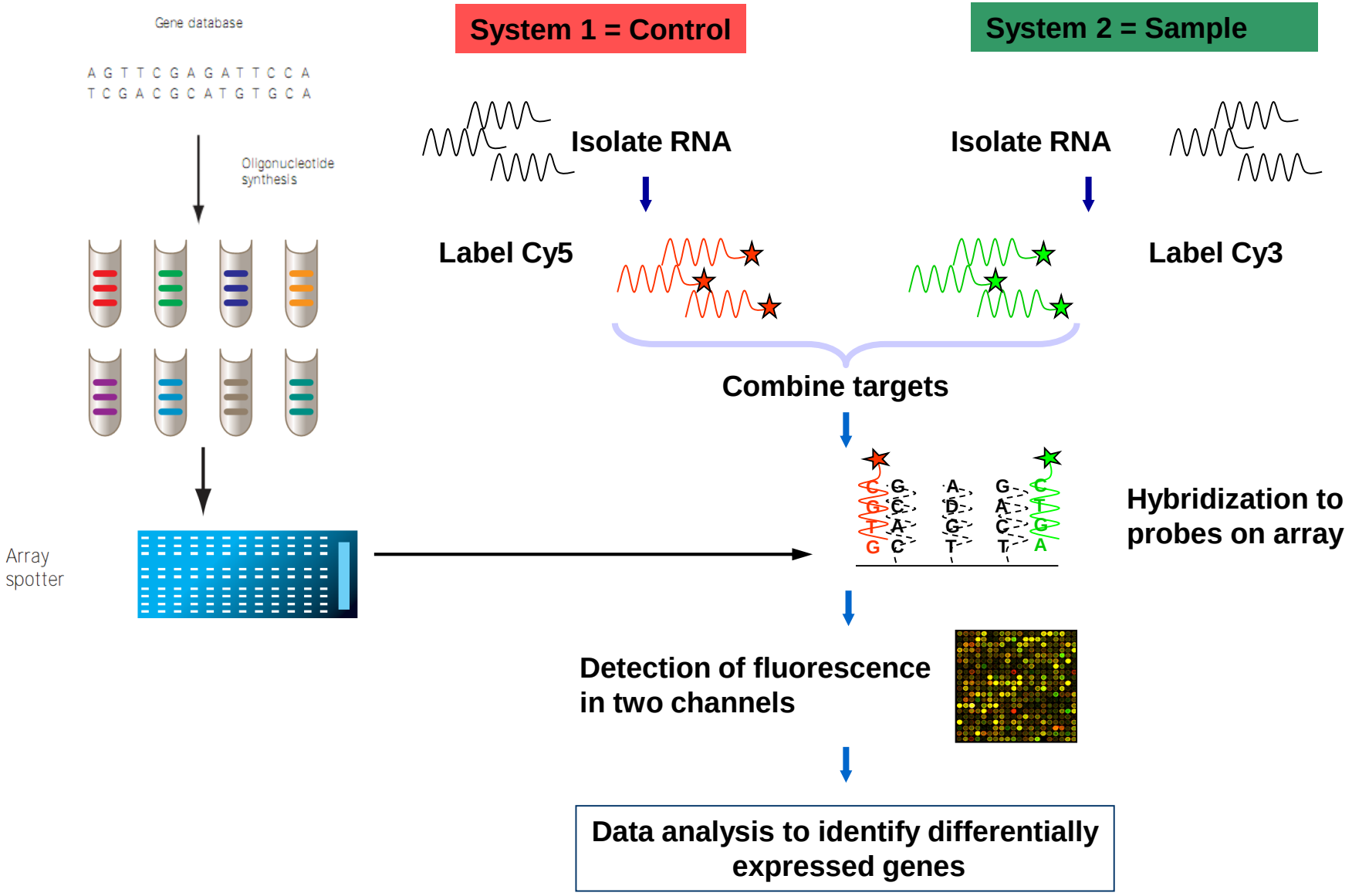
- light sensitive protecting groups, light + mask techique
- in situ combinatoric solid phase synthesis



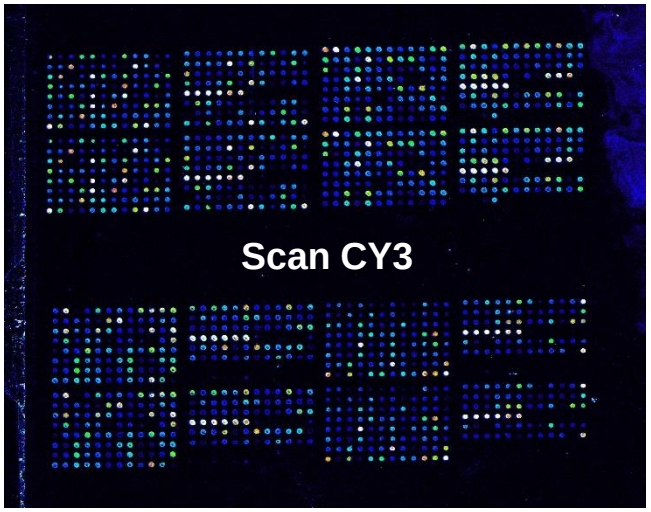
- incubation of activated areas with protected nucleotides

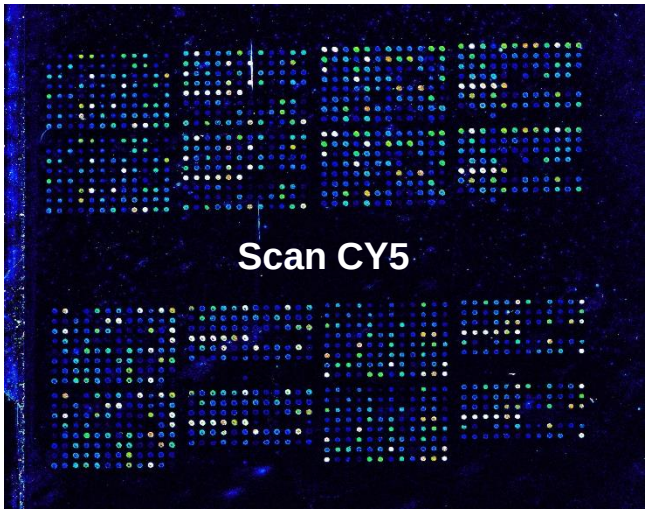
32 cycles (32 masks) → 65000 oligonucleotides (8 bases)

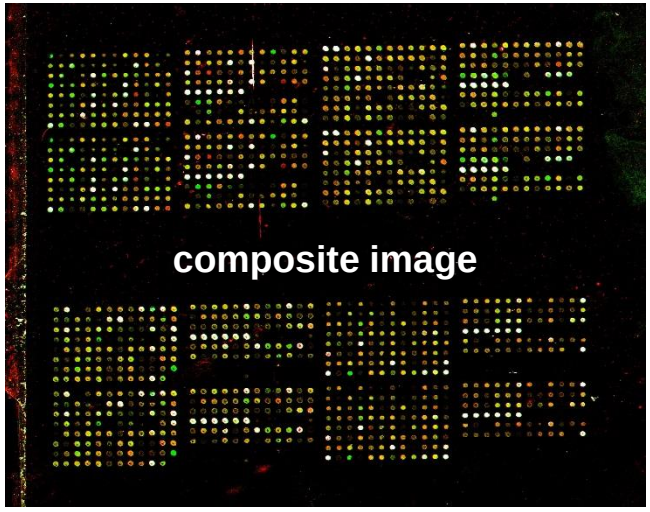
Sample Preparation - RNA

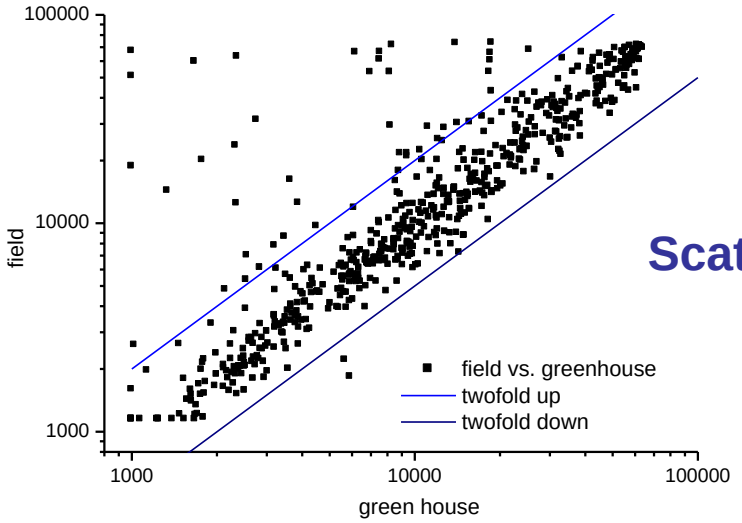


Detection → Two Color Experiment







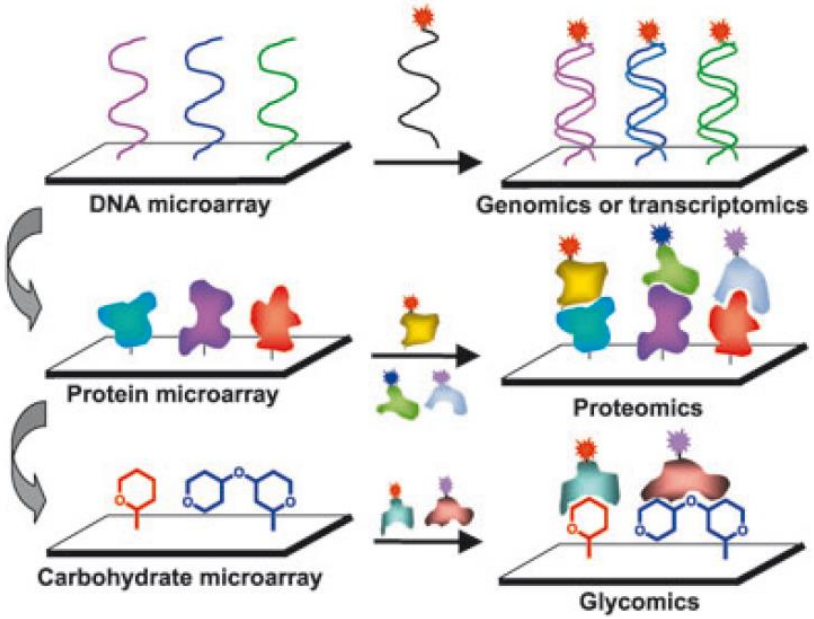


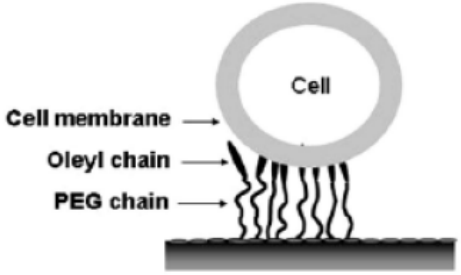
Scatter plot

Courtesy of Frédérique Pasquer (Institute of Plant Biology, Uni Zurich)

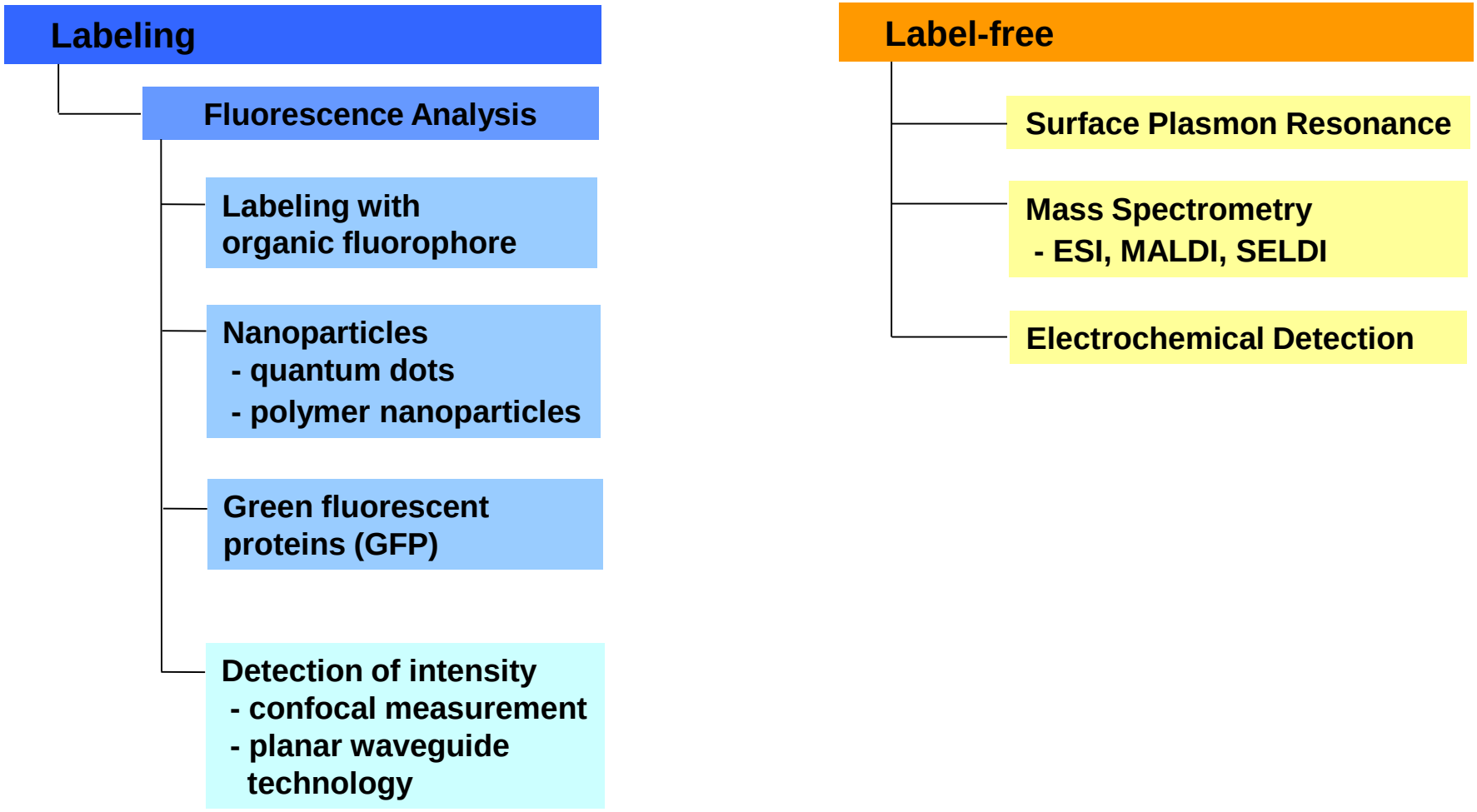
DNA, Protein, Peptide, Carbohydrate, Cell, Tissue, … Microarrays

- DNA- and oligonucleotide microarrays
  - **Probes**: ssDNA, oligonucleotides
  - **Targets:** ssDNA, ssRNA
  - Nucleic acid „chemistry“
- Protein and peptide microarrays
  - **Probes:** Any protein (e.g. antigen)
  - **Targets:** Any other protein (e.g. antibody)
  - Amino acid „chemistry“
- Others
  - **Cell / tissue microarrays**
  - Transfection microarrays
  - ....



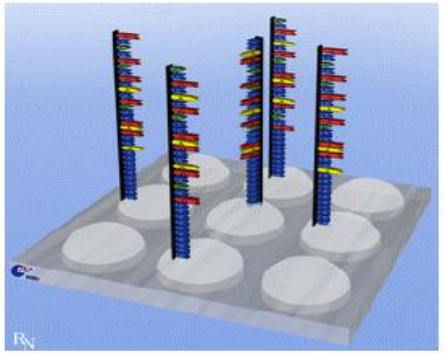


Detection Principles for Microarrays

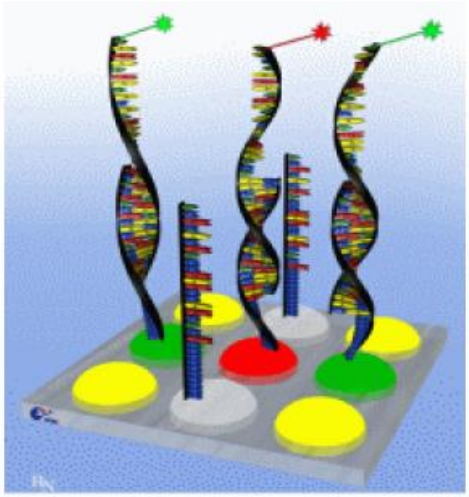


Detection Principles for Microarrays

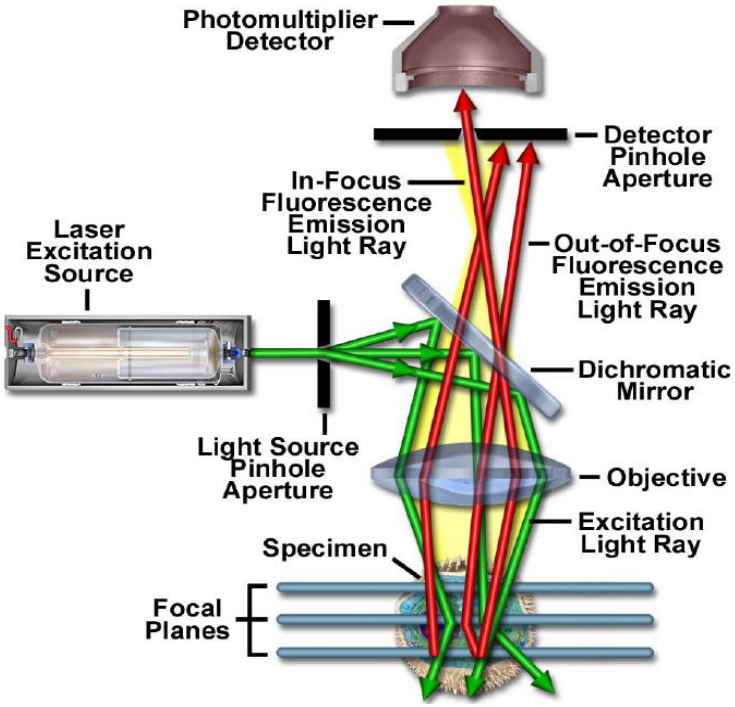
- Labeling of probe or target with fluorescent dye
  - Large amount of possible techniques known
  - Multiplexing possible (different deyes / colors)
  - Signal can be interpreted quantitatively (intensity ~ # hybridize molecules)
  - Fluorescent signal occures only where hybridisation has taken place



- Detection of fluorescent signal with optical reader
- Practical issues
  - Unspecific binding
  - Photobleaching
  - Quenching



Detection of Fluorescence using Principle of Confocal Microscopy



www.zeiss.com

Advantages

- **Single pixel laser excitation**
- **Very good background suppression**
  → high S/N ratio

Disadvantages

- **Limited by choice of excitation wavelenghts**
- **Focus depth of some 10 μm requires substrates of high planarity and exact positioning**
- **High hardware costs**

Using of Laser Scanners

Axon Genepix 4000B

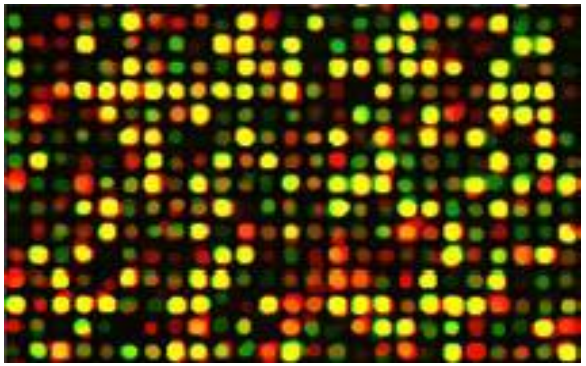
Typical Features of a Laser Scanner:

- **Excitation → Laser, e.g. solid state (488 nm), HeNe (543 nm, 594 nm, 633), Nd YAG (532 nm), 10-20 mW**
- **Detection → Photomultiplier tube or CCD camera**
- **Sensitivity → 0.02 – 1 fluorophor CY5 / mm^2**
- **Resolution → 5 - 100 μm**
- **Image format → 16 or 24 bit tif**

Advantage of Confocal Measurement

Planar supports

Confocal Scan



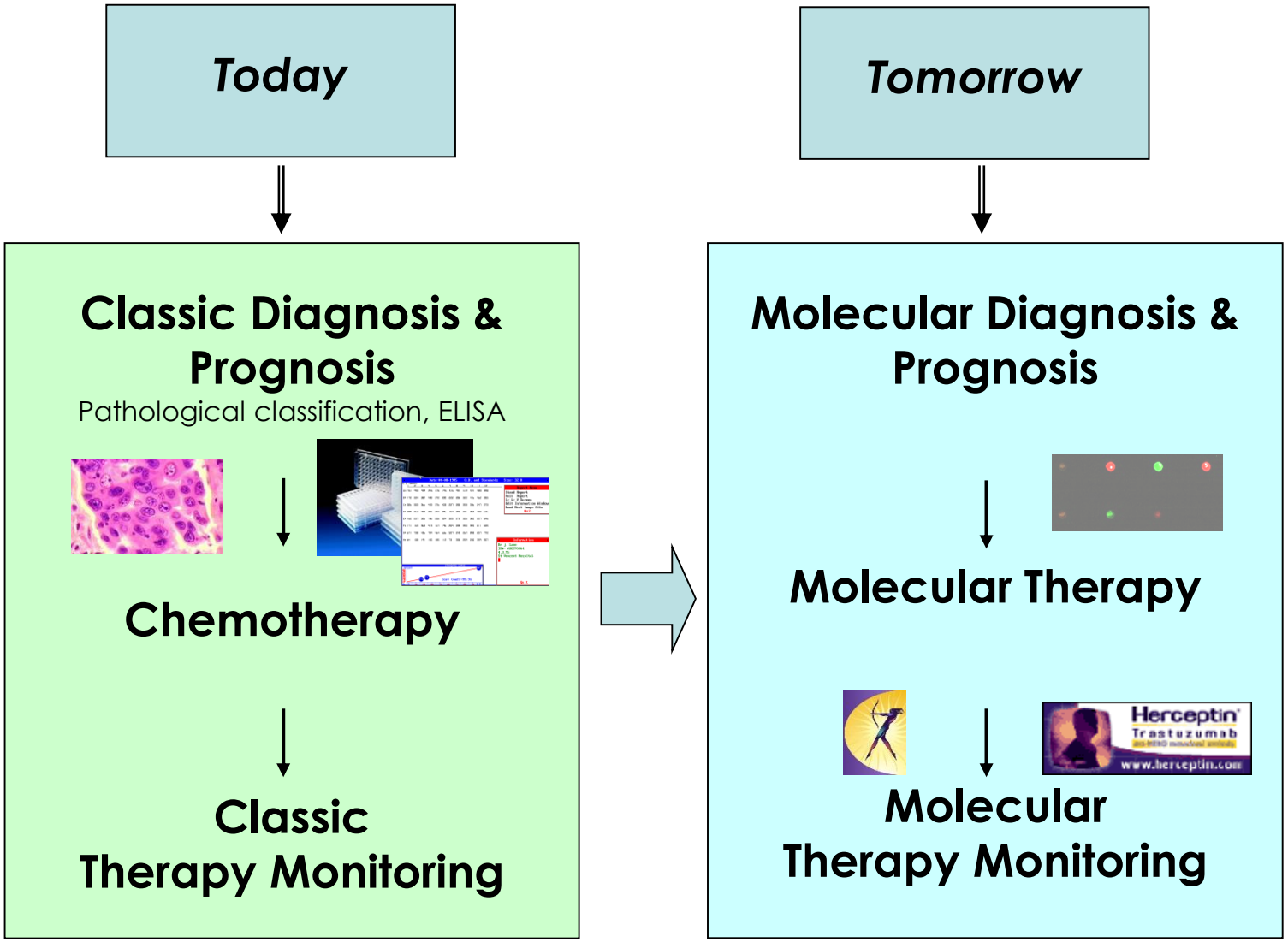
Non-Confocal Scan

www.tecan.com

⇒ **better signal to background ratio for planar surfaces and formats with 3D structure**

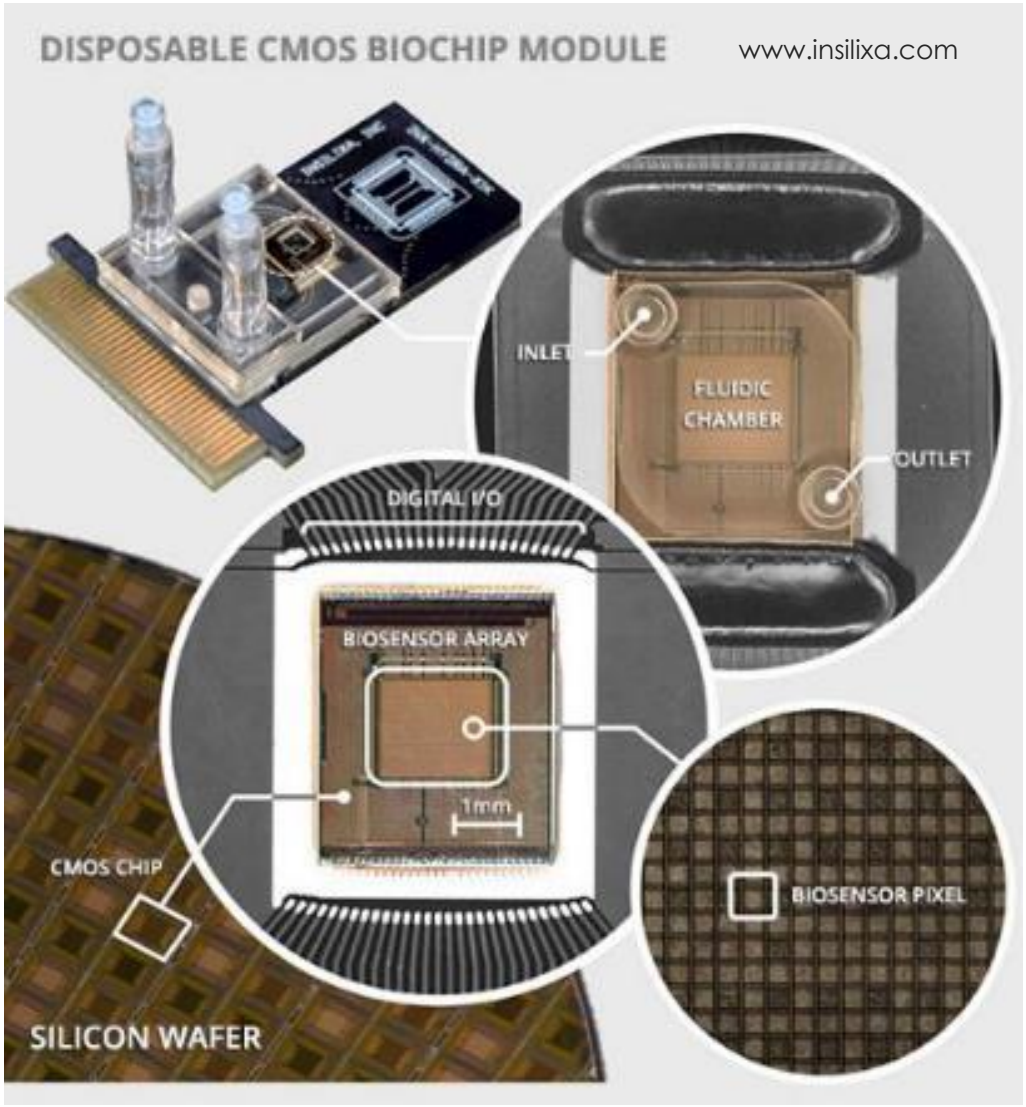
⇒ **especially for materials with intrinsic higher fluorescence background like polymers**

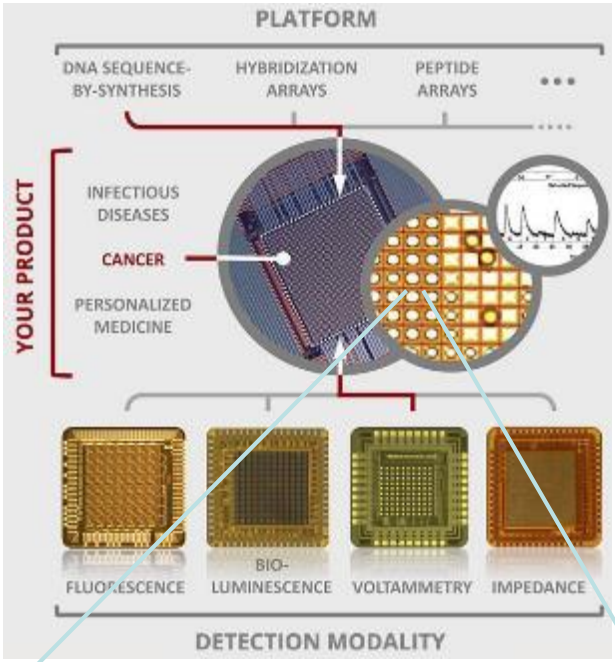
Microarrays - Clinic

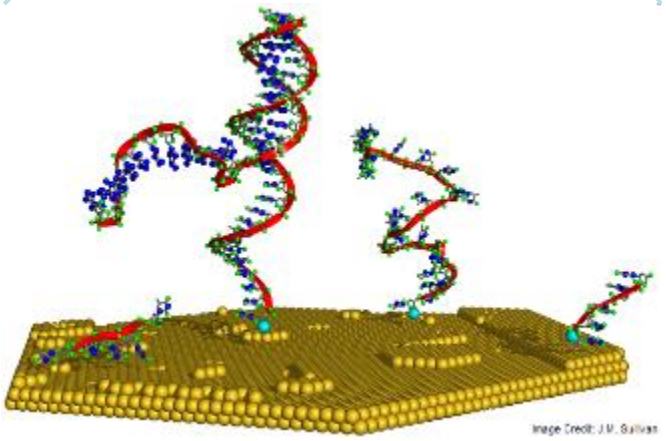


Biosensors

Biosensors Require Exact Loading of Sensor Fields







→ **Control of behavior of biomolecules attached to surface on a nanometer scale**

Biosensors

Biosensor → Combination of biological **recognition element** a **microfluidic chip** and a physical or chemical **transduces** to detect an analyte

Blood Glucose Measurement 85% of Market

Glucose Sensing - Basic Principles

Enzymes

- *Glucose Oxidase (GOx)*

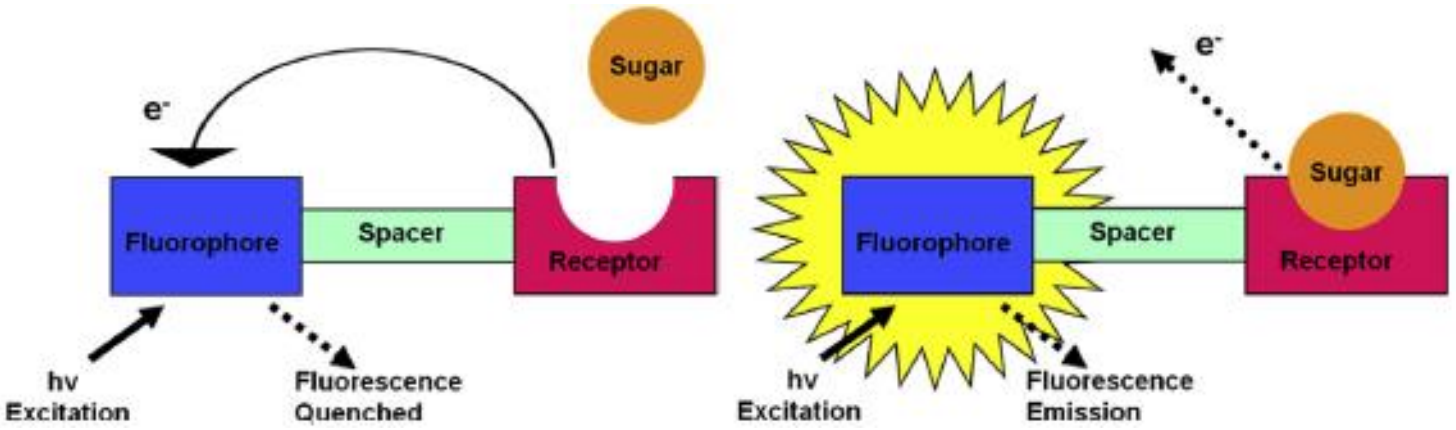
$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\mathbf{GOx - FADH_2 + O_2 \rightarrow GOx - FAD + H_2O_2}$$

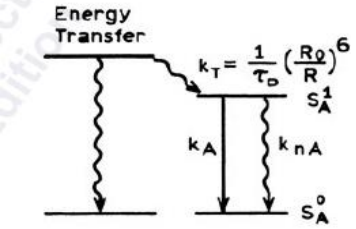
$$H_2O_2 \rightarrow 2H^{+} + O_2 + 2e$$

  - Used in biosensors
  - Easy to obtain, cheap
  - High selectivity, withstands greater extremes of pH, T, ions

Enzyme free

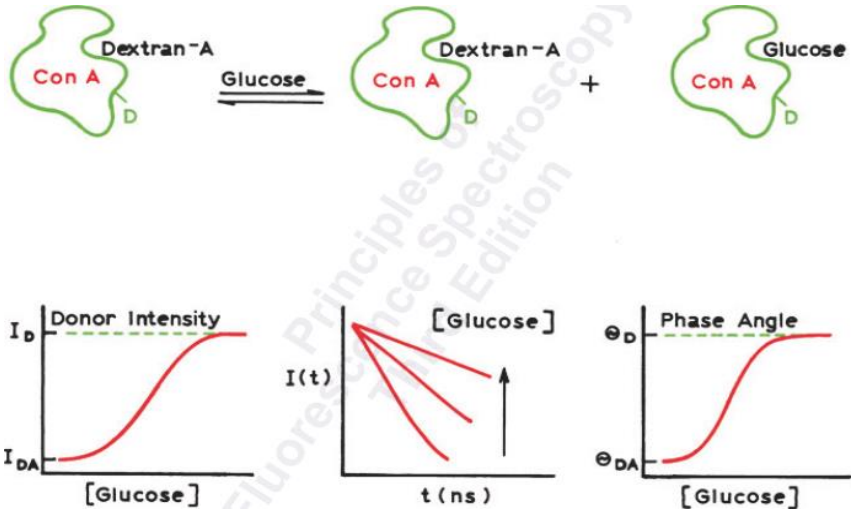


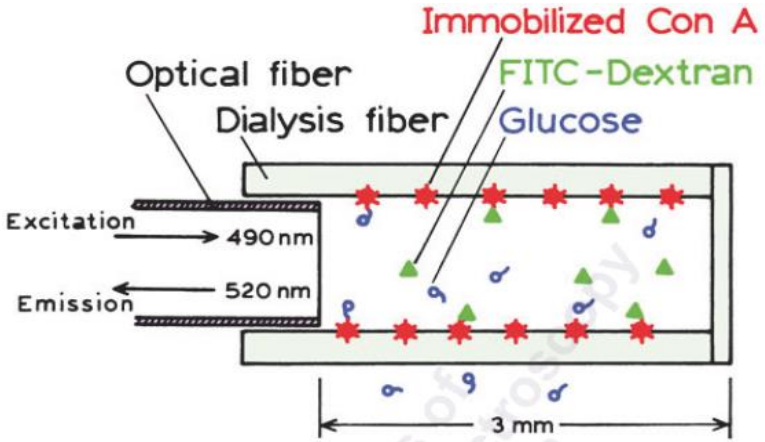
Sensing basierend auf FRET
→ Blutzuckerbestimmug



Glucose-Sensor

- Protein Concanavalin A (Con A) gelabelt mit Donor
- Glukose + konkurrierendes Polysaccharide (Dextran) gelabelt mit Akzeptor





→ Bindung von Dextran-A an Con-A → Abnahme Donor FL-Intensität und Lebensdauer
→ Bindung von Glucose → weniger Energietransfer, Zunahme FL-Signal vom Donor