EPFL Summer School on Nano-Bio-Sensing
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Introduction to Electronic DNA Microarrays

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Purpose of this Talk

Electronic and in particular CMOS-based bio-molecule sensor chips have attracted much attention during recent years.

Successful operation and development of such platforms requires to match and to understand the interdependencies of sensor principle, manufacturing processes, material sciences, CMOS extension, sensor interface circuitry, assembly techniques, fluidics, … .

Purpose of this talk is to provide an overview about electronic bio-molecule interfacing and detection techniques, considering
- related basics in the domain of biology
- various transducer approaches,
- CMOS processing extensions,
- circuit requirements and practical design aspects
- assembly and system issues

Outline (Part I)
Introduction to electronic DNA Microarrays

1. **Purpose of this Talk**
2. **Introduction to Genes and Genome, DNA, RNA, and Proteins**
3. **Microarrays - Basics and Applications**
4. **Functionalization Techniques**
5. **CMOS Integration**

Outline (Part II)
CMOS DNA Microarrays: Circuit and System Aspects

6. Electrochemical Readout Techniques
   - 6.1 Transducer Principles
   - 6.2 Potentiostatic Setup
   - 6.3 Design Examples Readout Circuitry
7. Further Readout Techniques
   - 7.1 Labeling-Based Approaches
   - 7.2 Labeling-Free Approaches
8. Assembly and Packaging
9. Conclusion

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Genome, Genes, Nucleic Acids, Proteins

Genome
synonym of the entirety of all genes of an organism

Gene
- classical genetics: hereditary disposition
- molecular genetics: functional intercept of DNA

Nucleic acids
- carrier of the full genetic information
- two types of nucleic acids:
  - **DNA** (Desoxyribonucleic acid)
    to *conserve and transfer* the genetic information by replication
  - **RNA** (Ribonucleic acid)
    to *express* the genetic information

Proteins
- essential for cell operation, significant contributions to entire cell metabolism
- various specific functions

Genes and Genome (Further Remarks)

Genes:

- genes play in concert!
  (i.e. the idea "a single gene codes for a single property" is wrong)
- distribution / density of genes on chromosomes (large macromolecules hosting the DNA and proteins) is inhomogeneous

Human genome:

- approx. 32,000 genes
- only 1.5 % of the entire genome are genes
- longest human gene approximately 2.4 Mb
- Human Genome Project: today 99% of gene containing part of human sequence identified with 99.99 % accuracy

Different species:

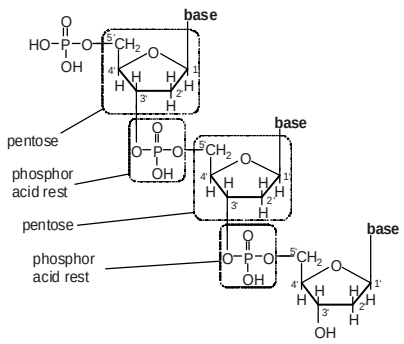
- density and number of genes show huge amount of variants
- number of chromosomes varies and is not proportional to the magnitude of the genome (humans: 46, carps: 104, flies (Drosophilidae): 8, mice: 40, pigs: 38, horses: 64, ...)

DNA, RNA, and Proteins

Basis structure and components	Stability
DNA	
• (usually) double-stranded structure → double helix with base pairs • information coded by means of four different bases: Guanine (G), Adenine (A), Cytosine (C), and Thymine (T)	• relatively stable • relatively easy to handle
RNA	
• single-stranded structure • information coded by means of four different bases Guanine (G), Adenine (A), Cytosine (C), and Uracil (U)	• not stable • short term copy of DNA information
Proteins	
• huge amount of different structures and shapes • information coded / function determined using 20 different types of amino acids	compared to DNA: • by far lower stability • more complex handling

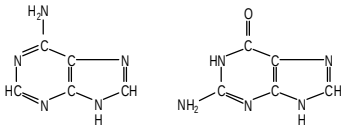
DNA Structure

Structure of a single strand



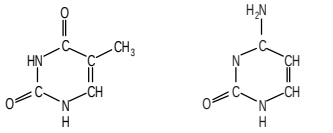
series of mononucleotides coupled by phosphor acid rests

DNA bases



Adenine (A) Guanine (G)

Purine bases



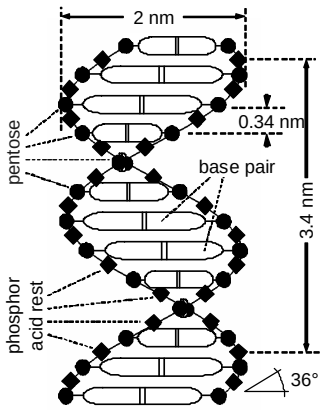
Thymine (T) Cytosine (C)

Pyrimidine bases

DNA Structure and Physical Properties

DNA double helix:

- DNA usually organized in double-stranded form
- Complementary (matching) base pairs:
  A-T (2 Hydrogen bridges)
  G-C (3 Hydrogen bridges)



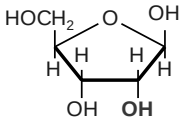
Physical properties of DNA:

- Negatively charged
- Mass of 1 nucleotide pair ≈ 1.1×10^{-21} g
- Further properties see figure
- Lengths:
  - salamander: ~ 9×10^{10} base pairs
  - corn: ~ 1.5×10^{10} base pairs
  - **humans: ~ 3.2×10^{9} base pairs**
  - tomato: ~ 7×10^{8} base pairs
  - E coli: ~ 5×10^{6} base pairs
  - simple viruses: > several 10 k
  - plasmids: 1...250 k

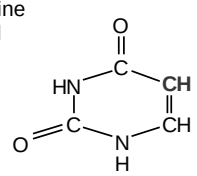
RNA
Structural Differences Compared to DNA

RNA

- **single**-stranded
- **pentose**

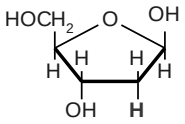


- bases:
  - Guanine
  - Adenine
  - Cytosine
  - **Uracil**

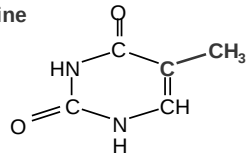


DNA

- **double**-stranded
- **pentose**



- bases:
  - Guanine
  - Adenine
  - Cytosine
  - **Thymine**



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DNA Detection

Distinguish:

- **DNA Sequencing**

  Determination of the order of bases (A, G, C, T) in DNA molecules. The sample consists of (a single species of) unknown DNA.

- **Hybridization Assays**

  Usually highly parallel investigation of a given sample concerning the amount of specific pre-defined DNA sequences.

DNA microarrays, frequently also simply referred to as DNA chips, are applied for the latter purpose.

DNA Microarray Chips

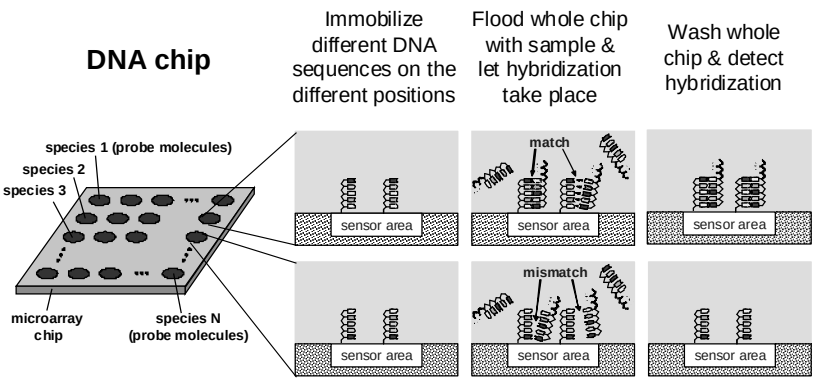
Purpose:
Highly parallel investigation concerning the presence / absence / quantitative amount of specific (pre-defined) DNA sequences in a given sample

Basic setup:
Slide ("chip") of the order mm^2 ... cm^2 made of glass / polymer material / Si

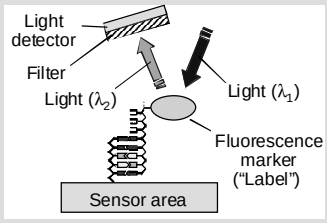
Most important applications:
- Genome research
- Drug development
- Medical diagnosis

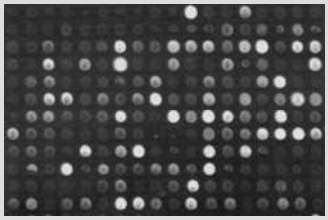
Application dependent requirements:
- Sensitivity / dynamic range
- Specificity

Basic Operation Principle of DNA Microarrays



Basic Operation Principle of DNA Microarrays
State-of-the-Art Optical Readout





Optical readout technique. Left: principle, right: typical result, overlay from a number of experiments using artificial color presentation.

- Principle:
  - target strands are labeled with fluorescence marker molecules
  - chip is illuminated with light of wavelength λ_1
  - entire chip is scanned by a camera system and fluorescence light (λ_2) is detected
- Most commercially available systems are based on this detection method
- Alternative optical technique: chemiluminescence

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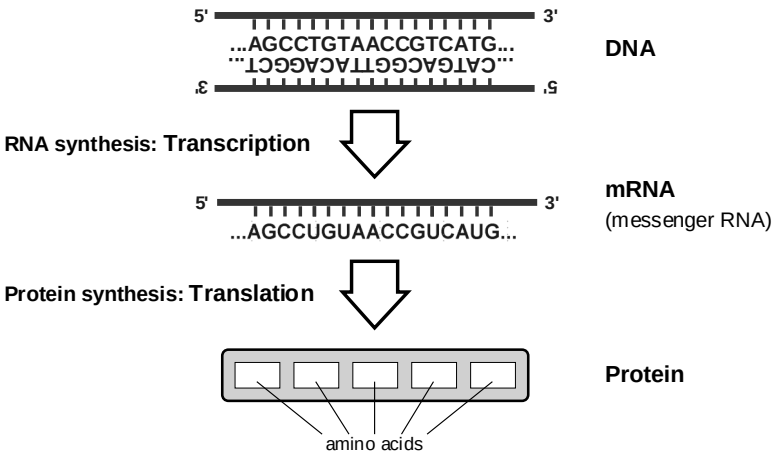
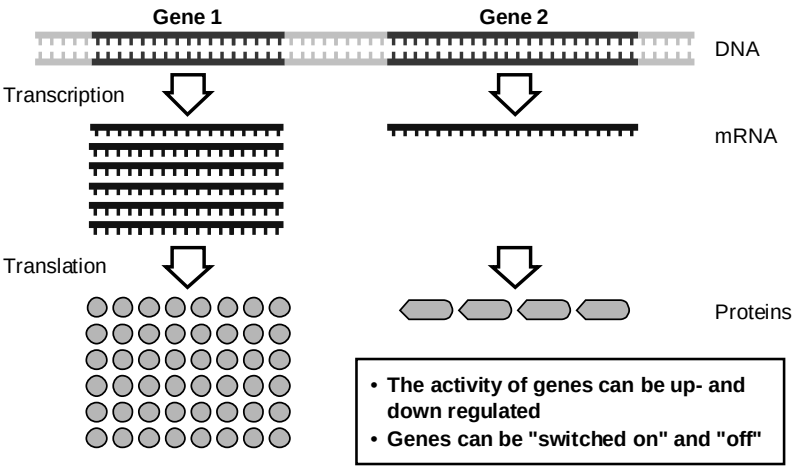
Gene Expression (I):
From DNA to Proteins – Basic Principle



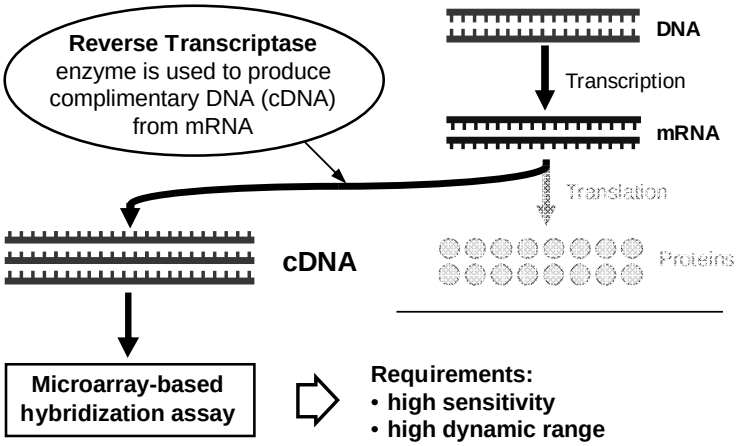
Gene Expression (II):
Up- and Down Regulation of Gene Activity



- **The activity of genes can be up- and down regulated**
- **Genes can be "switched on" and "off"**

Microarray Applications
Gene Expression Monitoring

Purpose e.g.: Monitoring the effect of drugs on gene expression



Microarray-based hybridization assay

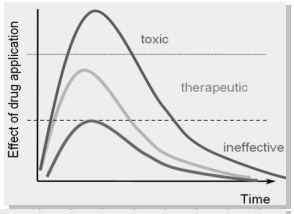
Requirements:
- **high sensitivity**
- **high dynamic range**

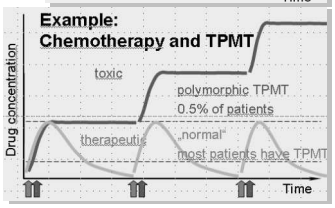
Microarray Applications
SNP detection (I)

- **SNP: Single nucleotide polymorphism**
- Some polymorphisms can be easily recognized: eye color, hair color, ...
- Other polymorphisms determine the way patients respond to drugs:
  - to achieve a pharmacological response, the drug has to reach a minimum concentration
  - drug concentrations are influenced by absorption, distribution, metabolism, and binding to the target protein.
- Today, physicians are unable to predict if an applied drug will have therapeutic effects and/or if adverse reactions will occur after a therapy is started.
- The idea of individualized medication is: "right drug, right dose, right patient"
- For this purpose, the patients SNPs must be determined

  +

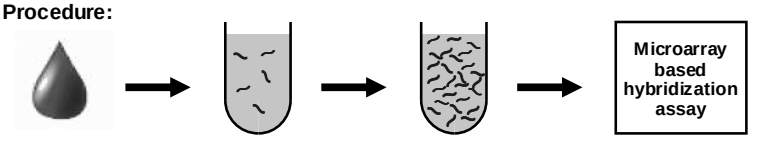
  the related knowledge (which SNPs reveal the required information?) must be generated





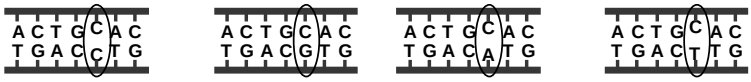
Microarray Applications
Example SNP detection

Procedure:



Sample (e.g. blood) → DNA isolation (lysis of cells etc) → DNA amplification (PCR) → DNA detection + experiment evaluation

Provide capability to distinguish



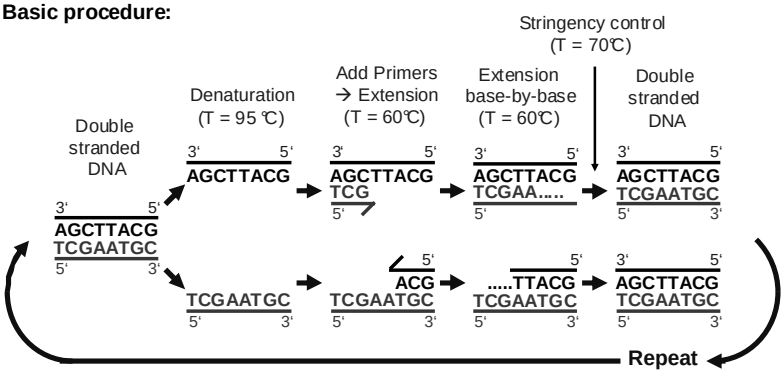
Single base pair mismatch | **Perfect match** | Single base pair mismatch | Single base pair mismatch

→ **Requirement: high specificity**

Excursus:
Polymerase-Chain Reaction (PCR) (I)

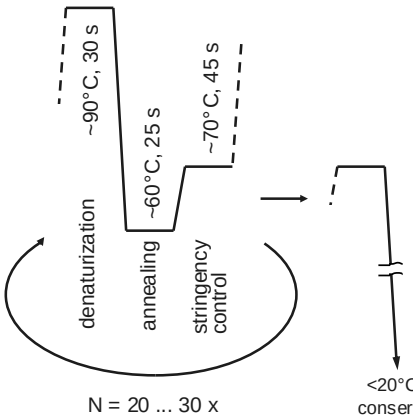
PCR used to amplify amount of available target molecules.

Basic procedure:



Excursus:
Polymerase-Chain Reaction (PCR) (II)

Typical temperature protocol:



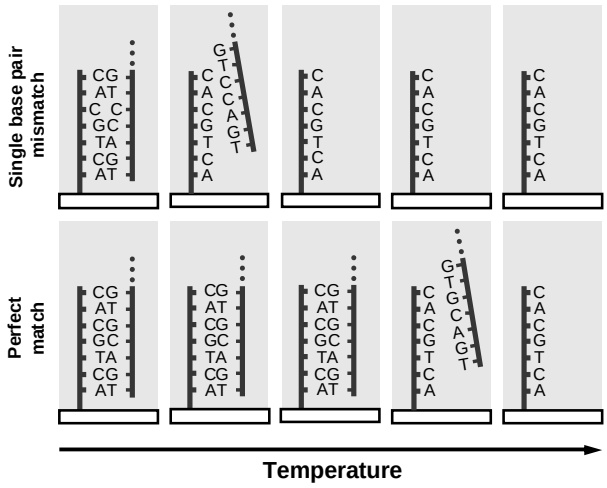
Remarks:

- Ideal PCR leads to a gain factor of 2^N
- In real cases, however, a gain < 2^N is achieved

Definitions, variants:

- Multiplex-PCR:
  PCR to amplify different target molecules in parallel
  → challenge:
  - find optimum conditions for all targets to be amplified
  - different gain for different targets possible
- Asymmetric PCR:
  amplification of one of the two strands only
  → gain << 2^N

Microarray Applications
Specificity



High specificity is achieved by subsequent measurements under increasingly unfavorable binding conditions, e.g. at increasing temperature.

Why Electronic Functionality?

State-of-the-art commercially available DNA microarrays

- mostly based on optical readout techniques
- mostly based on optically driven or mechanical functionalization techniques

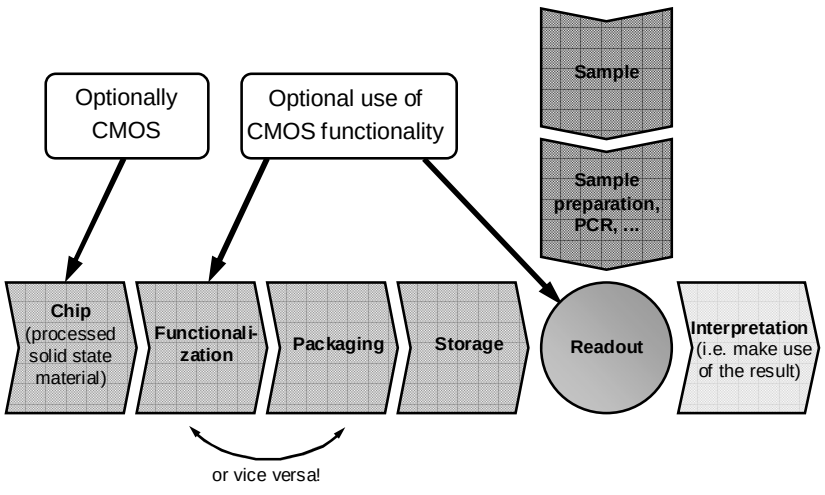
Opportunities provided by electronic techniques

- increased robustness
- increased user friendliness
- decreased system cost
- increased flexibility
- …

Requirement of large arrays:
("large" in this context: ≥ 10...100 sensor sites)

- CMOS integration mandatory

Entire Manufacturing / Application Chain of Microarrys



Electronic vs. Optical Detection
Independent Assay and Application Parameters

- **Point-of-Care Suitability**
  - requires small (handheld) devices
  - system solution gated by questions related to sample preparation
- **Real-Time Detection Capability**
  depends on dynamic behavior of assay (long target strands vs. short target strands), sample agitation, … , but not on transducer physics
- …

Basic Literature and Review Articles

- **E. M. Southern, "An improved method for transferring nucleotides from electrophoresis strips to thin layers of ion-exchange cellulose", Anal Biochem., 1974, November, 62(1), pp. 317-318.**
- **"The Chipping Forecast", Nature Genetics Supplement, Volume 21, Jan. 1999.**
- **http://www.nature.com/ng/chips_interstitial.html**
- **"DNA microarrays: a practical approach", M. Schena ed., Oxford University Press Inc., Oxford, UK, 2000.**
- **"Microarray Biochip Technology", M. Schena ed., Eaton Publishing, Natick, MA 01760, 2000.**
- **D. Meldrum, "Automation for genomics, sequencers, microarrays, and future trends", Genome Research, 2000, 10, pp. 1288-1303.**
- **F. Bier and J.P. Fürste, "Nucleic acid based sensors", in "Frontiers in Biosensorics I", F. Scheller, F. Schubert and J. Fedrowitz ed., Birkhäuser Verlag Basel/Switzerland, 1997.**
- **T. Vo-Dinh and B. Cullum, "Biosensors and biochips: advances in biological and medical diagnostics", Fresenius J. Anal. Chem., 2000, 366, pp. 540-551.**
- **P. Hegde, R. Qi, K. Abernathy, C. Gay, S. Dharap, R. Gaspard, J. E. Hughes, E. Snesrud, N. Lee, and J. Quackenbush, "A concise guide to cDNA microarray analysis", Biotechniques, 2000, September, 29(3), pp. 548-556.**

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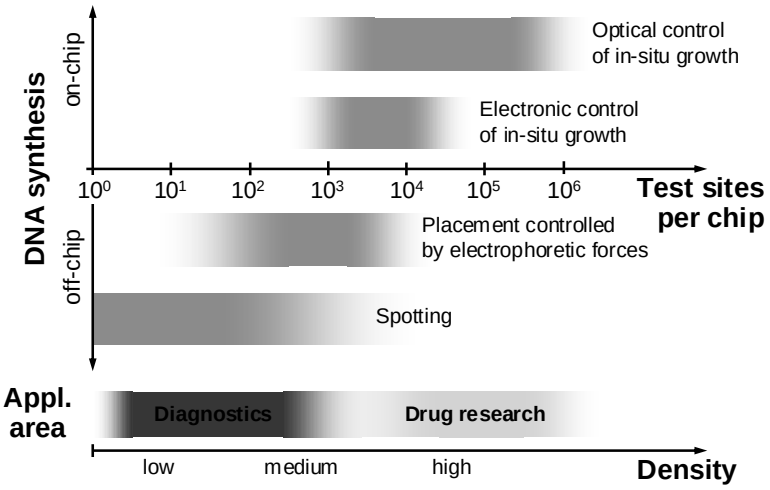
DNA Microarray Functionalization Techniques
... and Related Application Areas



Functionalization
Spotting (I)

Spotter contains:

- Pinhead with one or more pins, maneuverable in x-, y-, z- direction, positioning repeatability of order 10 µm,
- Reservoirs (e.g. microplates) with probe molecules in solutions + washing solution
- Chips to be functionalized
- Position recognition system

Procedure:

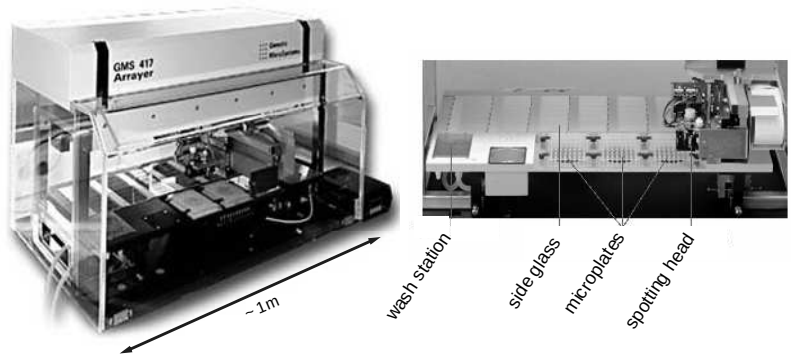
- Pins load solutions from reservoirs and deposit small volumes (of order 1 nl, various deposition techniques in use) at microarray target positions.

Pinhead with four pins

Stealth™ 48 pin printhead

Functionalization
Spotting (II)

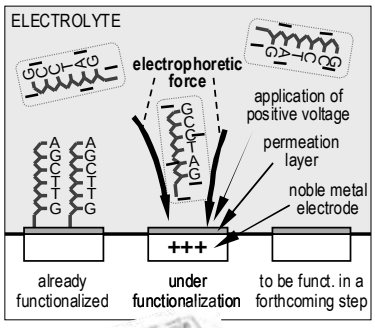
Example: Affymetrix Arrayer 417



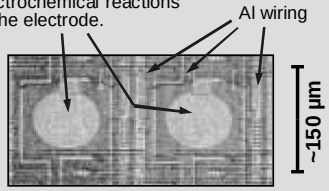
E. Zubritsky, Anal. Chem., 2000, December 1, 72(23), pp. 761A-767A.
V. G. Cheung et al., Nat Genet., 1999, January, 21(1 Suppl), pp. 15-19.
movies: www.bio.davidson.edu/courses/genomics/arrays/astart.html

Functionalization
Electrophoretically Driven Placement of DNA Probes

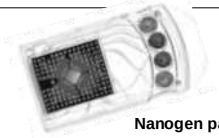
Direction of off-chip synthesized DNA receptor molecules to their on-chip target position controlled by electrophoretic forces.



Noble metal site with permeation layer to permit ion flow and to protect the DNA against damaging electrochemical reactions at the electrode.



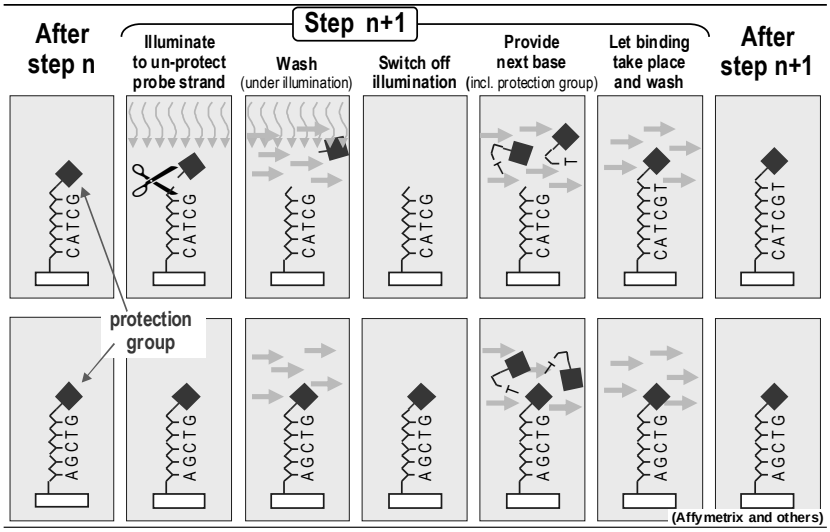
Sensor sites from a 20 x 20 Nanogen array using conventional optical readout.



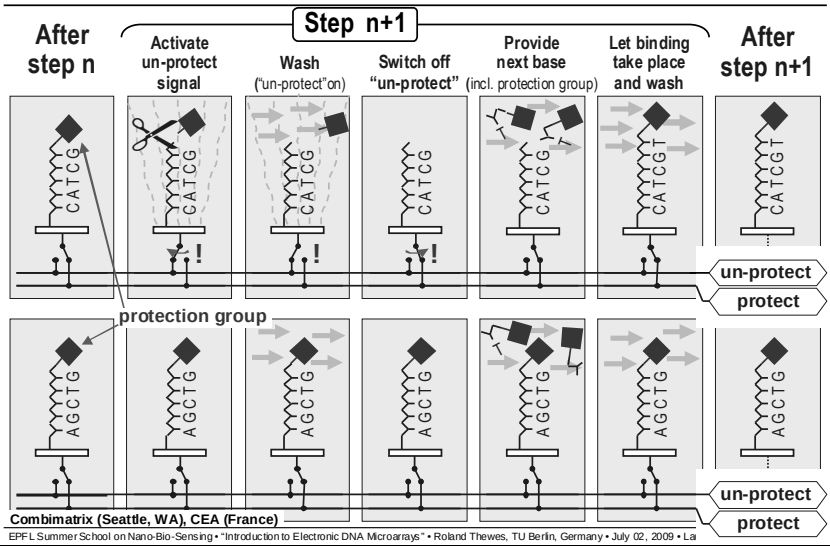
Nanogen package

T. Sosnowski et al, Proc. Natl. acad. Sci. USA, 1997
M. Heller, IEEE Eng. Medicine and Biology Magazine, 1996

Functionalization
Optically Driven In-Situ On-Chip DNA Synthesis



Functionalization
Electronically Driven In-Situ On-Chip DNA Synthesis



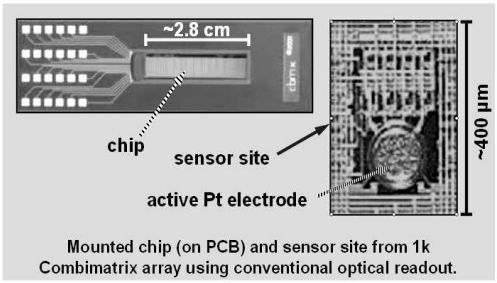
Combimatrix (Seattle, WA), CEA (France)

Commercially Available Platforms for In-Situ On-Chip DNA Synthesis

Affymetrix system:
optical synthesis / optical readout

Image courtesy of Affymetrix
www.affymetrix.com

Combimatrix system:
electrical synthesis / optical readout



Mounted chip (on PCB) and sensor site from 1k Combimatrix array using conventional optical readout.

Packaged 12k chip
Image courtesy of Combimatrix
www.combimatrix.com

K. Dill et al., Anal. Chim. Acta, 2001
K. Dill et al., J. Biochem. Biophys. Methods, 2004

CMOS Requirements in Case of Chips Using Electronically Driven Functionalization

- Introduction of noble metal electrodes / Extension of standard CMOS processes
- Provision of *low-frequency* logic circuitry
- Handling & switching of *large bias signals* to operate the electrodes

→ *relaxed requirements* concerning CMOS circuit design and CMOS process performance in case CMOS functionality is used for functionalization purposes only

→ requirements concerning *electrical readout* more challenging!

Outline (Part I)
Introduction to electronic DNA Microarrays

1. **Purpose of this Talk**
2. **Introduction to Genes and Genome, DNA, RNA, and Proteins**
3. **Microarrays - Basics and Applications**
4. **Functionalization Techniques**
5. **CMOS Integration**

Extended CMOS Process Options
Required for Electronic DNA Microarrays

Frequently used approach:
CMOS + noble metal

+

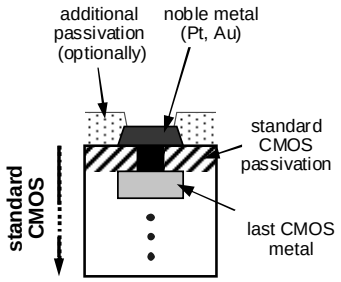
various application
specific extensions

+

various application
specific alternatives

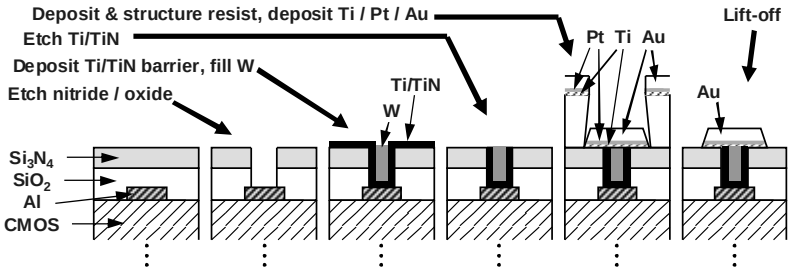
+

Flip-Chip solutions
with sensor chip + standard CMOS chip



Extended CMOS Process Options
Example: Au on Standard 0.5µm, 6" CMOS Process

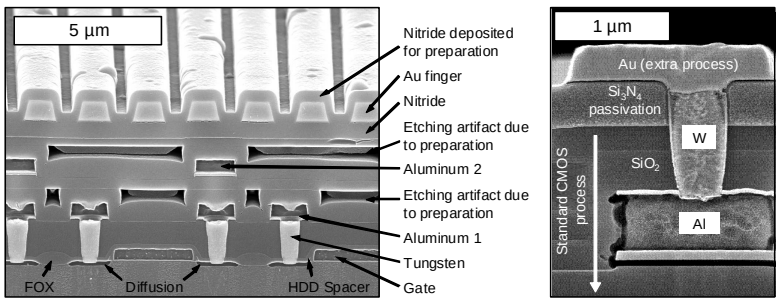
Backend process flow:



F. Hofmann et al., IEDM 2002

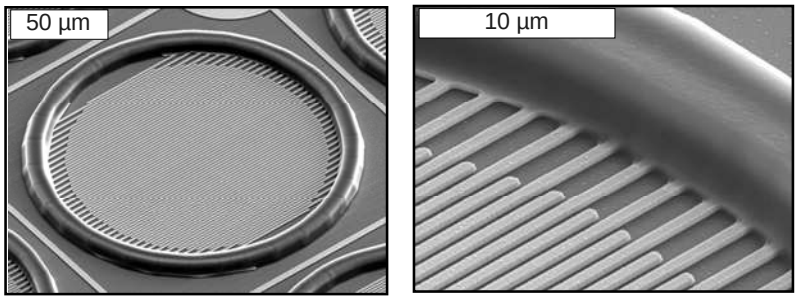
Extended CMOS Process Options
Example: Au on Standard 0.5µm, 6" CMOS Process

Cross section SEM photographs:

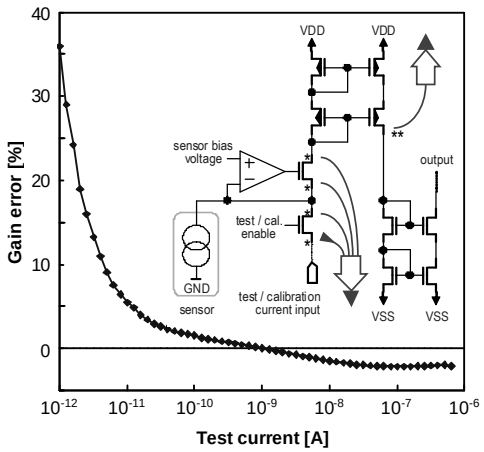


Extended CMOS Process Options
Example: Au on Standard 0.5µm, 6“ CMOS Process

SEM Photographs of interdigitated sensor gold electrodes:



Example CMOS + Au Processing
Device / Circuit Properties after Au Processing



Simple test circuit with test input used for 100-fold current gain of sensor current.

Specified sensor current:

10^{-12} A – 10^{-7} A

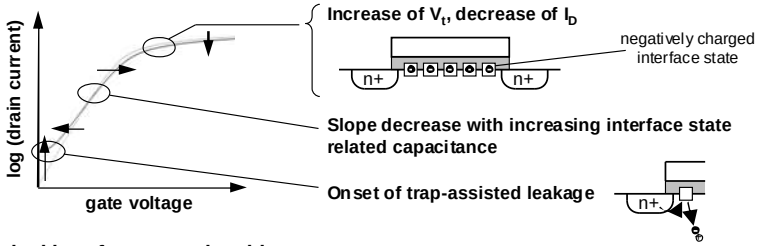
Insufficient behavior (high leakage currents) due to huge interface state density of > 10^{11} cm^{-2}

Excursus:
Gate Dielectric Interface States

Gate dielectric interface states:

- “dangling bonds” at the gate dielectric-to-silicon interface
- can capture and release charge carriers depending on the local Fermi level
- donor and acceptor type interface states

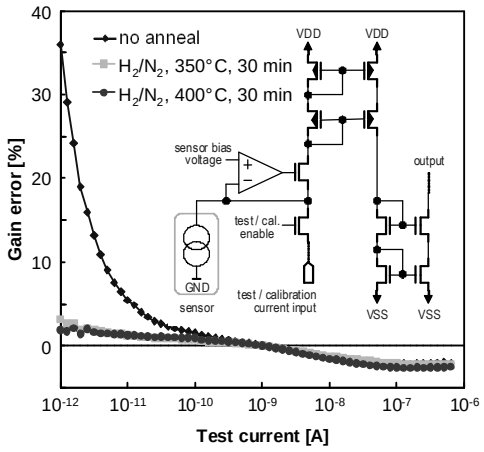
Effect of increased interface state density on MOSFET I/V characteristics:



Typical interface state densities:

- good, i.e. relatively low: ≤ 10^{10} cm^{-2}
- fair, still sufficient in many processes and applications: several 10^{10} cm^{-2}
- bad, i.e. in many applications too high: ≥ 10^{11} cm^{-2}

Example CMOS + Au Processing
Device / Circuit Properties after Extra Annealing Steps



Simple test circuit with test input used for 100-fold current gain of sensor current.

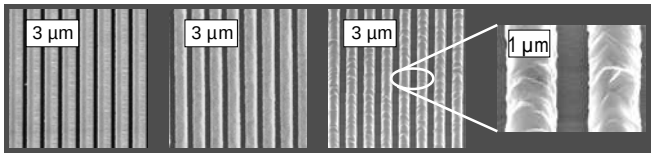
Specified sensor current:

10^{-12} A – 10^{-7} A

Sufficient behavior after additional H_2/N_2 annealing step after Au processing

Example CMOS + Au Processing
Final Process Window (Frontend + Backend)

	square resistance Au lines [mΩ/square]	resistance via holes (Al to Au) [mΩ]	square resistance Al 2 lines [mΩ/square]	interface state density [1/cm²]
CMOS only (i.e. without Au process)	-	-	-	~ 10^{10}
CMOS + Au process, no anneal	48	370	79	~ 2×10^{11}
CMOS + Au process, N_2/H_2 anneal with 350°C, 30 min	51	360	76	< 10^{10}
CMOS + Au process, N_2/H_2 anneal with 400°C, 30 min	61	340	74	< 2×10^{9}



no annealing **350°C, 30 min** **400°C, 30 min**

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CMOS Integration of Required Extra Processes
General Conclusion

- Extra processes are usually provided as post-CMOS backend extensions
- The related materials are often not compatible with CMOS line requirements
  → contamination issues must be considered
- Specific "post-post-CMOS" processing steps may be required to maintain CMOS performance + sensor performance
  → a suitable process window must be identified

Outline (Part II)
CMOS DNA Microarrays: Circuit and System Aspects