Introduction to CMOS Bio Sensor Arrays:
Electrical Specifications, CMOS Processing,
Circuit and System Design

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Purpose of this Tutorial

The achievements of Microelectronics
have had and will also in the future have an essential impact on our way of life.

Bio-technology and life sciences
are considered to be candidates that may trigger or already have triggered a similar
revolution as compared to the invention of the transistor some 50 years ago.

Purpose of this tutorial
is to give an introduction highlighting opportunities and challenges which arise
when these disciplines meet. In particular, an overview about the current status
of CMOS-based approaches for in-vitro applications is given considering
- basic operating principles and applications,
- required CMOS processing,
- electrical specifications and related circuit requirements,
- examples of successfully operated integrated circuit in this area.
Outline

1. Introduction and Purpose of this Tutorial
2. DNA and Protein Microarray Chips
   2.1 Basic Operation Principle and Applications
   2.2 Functionalization
   2.3 CMOS Integration
   2.4 Electrochemical Readout
      2.4.1 Detection Principles
      2.4.2 Potentiostatic Setup
      2.4.3 Readout Circuits
   2.5 Label-Free Approaches
      2.5.1 Quasi Labeling-Free Electrochemical Approaches
      2.5.2 Impedance-Based Sensors
      2.5.3 Gravimetric Sensors
   2.6 Packaging Aspects
3. Cell-Based Sensor and Actuator Chips
   3.1 Cell Manipulation and Cell Sorting
   3.2 Nerve Cell and Neural Tissue Interfacing
4. Further CMOS-/Si-Related Applications
5. Summary

2. DNA and Protein Microarray Chips

2.1 Basic Operation Principle and Applications
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 mm² ... cm² made of glass / polymer material / Si

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

Application dependent requirements:
• Sensitivity / dynamic range
• Specificity

DNA and Proteins (I)

DNA (Desoxyribonucleine acid):
• contains the full genetic information
• transfers genetic information by replication

Structure of a single strand:
series of mononucleotides coupled by phosphor acid rests

DNA bases:

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Adenine (A)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Guanine (G)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Thymine (T)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>Cytosine (C)</td>
</tr>
</tbody>
</table>

Purine bases
Pyrimidine bases
DNA and Proteins (II)

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 \( \approx 1.1 \times 10^{-21} \text{ g} \)
- Further properties see figure
- Lengths:
  - humans: ~ 3 \times 10^9 \text{ base pairs}
  - tomato: ~ 7 \times 10^8 \text{ base pairs}
  - E coli: ~ 6 \times 10^8 \text{ base pairs}
  - simple viruses: > several 10 k
  - plasmids: 1...250 k

Proteins:
- essential for cell operation, significant contributions to entire cell metabolism, various specific functions
- more complex to handle as compared to DNA molecules
- in part by far larger as compared to DNA molecules
Entire Manufacturing / Application Chain of Microarrays

Opportunities to operate a CMOS ASIC

or vice versa!

Why Electronic Readout?

State-of-the-art commercially available DNA microarrays:

- optical readout by labeling the target strands with fluorescence marker molecules

Opportunities provided by fully electronic readout techniques:

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

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

<table>
<thead>
<tr>
<th></th>
<th>passive electronic DNA chips</th>
<th>active electronic DNA chips</th>
</tr>
</thead>
<tbody>
<tr>
<td>density test sites per chip</td>
<td>low (of order 10 (+/-))</td>
<td>medium ... high (≥ 100 (+/-))</td>
</tr>
<tr>
<td>costs per chip</td>
<td>low</td>
<td>increased processing costs:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CMOS processing costs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- process to provide transducer elements must be compatible to CMOS process</td>
</tr>
<tr>
<td></td>
<td>approximately constant</td>
<td>decrease with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- increasing number of test sites per chip</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- increasing number of required data points per investigation</td>
</tr>
<tr>
<td>electrical performance</td>
<td>medium</td>
<td>high</td>
</tr>
<tr>
<td>electronic signal integrity</td>
<td>limited robustness</td>
<td>- by far increased robustness</td>
</tr>
<tr>
<td></td>
<td>- loss of signal integrity at high test site count per chip</td>
<td>- independent of number of test sites per chip</td>
</tr>
</tbody>
</table>

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2. DNA and Protein Microarray Chips

2.2 Functionalization
DNA Microarray Functionalization Techniques
... and related application areas

Functionalization by Spotting

Principle:

Typical technical realization:
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
- Optionally: 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

Example:
Affymetrix Arrayer 417

Example: Pinhead with four pins
**Functionalization**

by electrophoresis driven movement of off-chip synthesized DNA receptor molecules to their on-chip target position

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

CMOS requirements:

- provision of low-frequency logic circuitry
- handling & switching of large bias signals to operate the electrodes

Optically Driven In-Situ On-Chip DNA Synthesis *

* Principle used by Affymetrix, NimbleGen, FeBiT

**Step n+1**

<table>
<thead>
<tr>
<th>After step n</th>
<th>Wash</th>
<th>Switch off illumination</th>
<th>Provide next base (incl. protection group)</th>
<th>Let binding take place and wash</th>
<th>After step n+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATCG</td>
<td>CATCG</td>
<td>CATCG</td>
<td>CATCG</td>
<td>CATCG</td>
<td>CATCG</td>
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<tr>
<td>protection group</td>
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</tr>
<tr>
<td>ACGTG</td>
<td>ACGTG</td>
<td>ACGTG</td>
<td>ACGTG</td>
<td>ACGTG</td>
<td>ACGTG</td>
</tr>
</tbody>
</table>
Electrically Driven In-Situ On-Chip DNA Synthesis *

Step n+1

After step n

Activate un-protect signal
Wash (under 'un-protect' switched on)
Provide next base (incl. protection group)
Let binding take place and wash

After step n+1

un-protect
protect

un-protect
protect


Functionalization

by electronic in-situ synthesis on-chip

Mounted chip (on PCB) and sensor site from 1k CombiMatrix array using conventional optical readout. K. Dill et al., Anal. Chim. Acta, 2001
K. Dill et al., J. Biochem. Biophys. Methods, 2004

CMOS requirements:

- provision of low-frequency logic circuitry
- handling & switching of large bias signals to operate the electrodes
2. DNA and Protein Microarray Chips

2.3 CMOS Integration

Extended CMOS Process Options
Required for Electronic DNA Microarrays

Frequently used approach: CMOS + noble metal + … :

Note:
- For electronic functionalization purposes, mostly logic + little analog functionality is required
- For electronic readout, sufficient analog CMOS performance is required
Extended CMOS Process Options

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

Backend process flow:
- Deposit & structure Lack, deposit Ti / Pt / Au
- Etch Ti/TiN
- Deposit Ti/TiN barrier, fill W
- Etch nitride / oxide
- Lift-off

SEM photos:
- Cross section
- Top view

Example CMOS + Au Processing

Device / Circuit Properties after Au Processing

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

Specified sensor current:
- $10^{-12}$ A - $10^{-7}$ A

Insufficient behavior (high leakage currents) due to huge interface state density of $>10^{11}$ cm$^{-2}$
**Example CMOS + Au Processing**

Device / Circuit Properties after Au Processing + Extra Annealing Steps

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

Specified sensor current:

\[ 10^{-12} \text{ A} - 10^{-7} \text{ A} \]

**Definition of a Final Process Window**

Considering frontend + backend parameters

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

Specified sensor current:

\[ 10^{-12} \text{ A} - 10^{-7} \text{ A} \]
2. DNA and Protein Microarray Chips

2.4 Electrochemical Readout

2.4.1 Detection Principles

Standard Coulometric Approach

Three-Electrode-System

- Electrochemical label molecule
- Electron transfer from label
- Displacement current through double-layer capacitance $C_{dl}$

Basic setup

$\int v_{\text{step}}(t) \, dt$ (potentiostat)

Alternative: step applied by potentiostat → in particular interesting for operation of arrays

Voltage step leads to oxidation (or reduction) of the label if present at the considered test site

Result (schematic plot)
Signal-to-Offset-Ratio
Coulometric Approach / Three-Electrode-System

Signal-to-Offset-Ratio \( SOR = \frac{\text{charge from label molecules}}{\text{charge from double-layer capacitance}} = \frac{n \times q 	imes D_{\text{probe}}}{V_{\text{step}} \times C_{\text{dl}}} \)

with \( n \) = amount of electrons per oxidation/reduction per label
\( q \) = elementary charge \((= 1.6 \times 10^{-19} \text{ As})\)
\( D_{\text{probe}} \) = density of probe molecules on the test site
\( V_{\text{step}} \) = amplitude of voltage step
\( C_{\text{dl}} \) = areal double-layer capacitance

Application of reasonable values:
\( n = 1, D_{\text{probe}} = (10 \text{ nm})^2, V_{\text{step}} = 300 \text{ mV}, C_{\text{dl}} = 20 \mu \text{F cm}^{-2} \) \( \Rightarrow SOR = 2.7 \% \)

Requirements / guidelines for practical applicability:
- Decrease \( C_{\text{dl}} \):
  \( \Rightarrow \) Introduce electrode surface blocking layer after probe molecule immobilization
- Increase \( n \) (by using other / modified label molecules)
- Increase \# label molecules per target
- Use labels with decreased values of \( V_{\text{step}} \)

Example:
Typical (Large Area Electrode) Chronocoulometric Measurement

\( Q_{\text{total}} / \text{area} = n \times q \times D_{\text{probe}} + V_{\text{step}} \times C_{\text{dl}} \times \alpha \rightarrow \text{of order} 10 \text{ nC mm}^{-2} \)

with \( n = 1 \ldots 5 \) (amount of electrons per oxidation/reduction per label)
\( q = 1.6 \times 10^{-19} \text{ As} \) (elementary charge)
\( D_{\text{probe}} = (10 \text{ nm})^2 \) (density of probe molecules on the test site)
\( V_{\text{step}} = 100 \ldots 300 \text{ mV} \) (amplitude of voltage step)
\( C_{\text{dl}} = 10 \ldots 40 \mu \text{F cm}^{-2} \) (areal double-layer capacitance)
\( \alpha = 15 \ldots 25 \% \) (double-layer capacitance reduction factor due to blocking layer)
Redox-Cycling Approach
4-Electrode System with Interdigitated Working Electrodes

- Target DNA molecule labeled with enzyme molecule (not electrochemically active)
- Application of an additional substrate, which is not electrochemically active in the provided form, but can be cleaved by the enzyme into electrochemically active sub-species
- Application of positive and negative voltages of order ±few 100 mV at neighboring electrodes starts redox-cycling (i.e. reduction and oxidation) process

Redox-Cycling Sensor Signals:
Properties and Typical Measured Data

- characterization time: seconds
- evaluated signal: $\frac{\partial \text{current}}{\partial \text{time}}$
  (since the absolute values of the current may be affected by time-independent artifact)
- current range & required resolution: 1 pA ... 100 nA
**Sensor Principle Redox-Cycling**

**Chemical Reactions (exemplary)**

**Process at the label:**

\[
\text{NH}_2 \xrightarrow{\text{OPO}_3^{2-}} \text{PO}_3^{2-} + \text{OH}^- \\
\text{p-Aminophenylphosphate} \quad \text{Alkaline Phosphatase} \quad \text{p-Aminophenol}
\]

**Substrate**

**Label**

**Electrochemically active molecule**

**Process at the electrodes:**

\[
\text{HO-} \xrightarrow{\text{NH}_2} \xrightarrow{-2e^-, -2H^+} \text{O} \xrightarrow{\text{NH}} \text{Oxyd}: \text{Quinoneimine}
\]

**Red:** p-Aminophenol

**Ox:** Guinoneimine

**Comparison:**

**Important Properties of 3- and 4-Electrode Systems**

<table>
<thead>
<tr>
<th></th>
<th>3-electrode systems</th>
<th>4-electrode systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode blocking to decrease value of electrode-to-electrolyte capacitance</td>
<td>required</td>
<td>no</td>
</tr>
<tr>
<td>Amount of chemical steps / substrates involved</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Dynamic range</td>
<td>low ... medium</td>
<td>high</td>
</tr>
<tr>
<td>Electrochemical crosstalk</td>
<td>no</td>
<td>possible</td>
</tr>
</tbody>
</table>
Further Labeling-Based Approaches

- **Gold bead labeling + Silver precipitation**
  - probe molecules are immobilized on isolating layer between electrodes
  - target DNA molecule are labeled with Au beads
  - after hybridization, Ag solution is applied to the sample
  - Au beads (present at positions with successful hybridization) act as seed positions to form a conductive Ag layer
  - Decrease of ohmic resistance reveals sites with matching DNA
  - Most recent publications use optical attenuation instead

  (J. Li et al., IEDM 2004)

- **Magnetic bead labeling**
  - target DNA molecule are labeled with magnetic nano-particles
  - after hybridization, magnetic properties are evaluated at the respective sites (e.g. using GMR sensors)
  - today: various promising proof-of-principles (using post-processed PCBs and other substrates), but no CMOS integration
  - R&D on-going, driven by a number of various groups in the world

  (M. Xue et al., IEDM, 2002, and ISSCC, 2003)

2. DNA and Protein Microarray Chips

2.4 Electrochemical Readout

2.4.2 Potentiostatic Setup
Potentiostatic Setup

Purpose of a potentiostatic setup:
Control / regulation of electrolyte potential

Setup / Configuration:

Parameters:
\[ R_{XX-XX}, C_{XX-XX} : \]
depend on
- electrolyte properties (concentration, compounds, ...)
- distances between respective electrodes

\[ R_{XE}, C_{XE} : \]
depend on
- properties of electrolyte and electrode material
- \( C_{XE} \) electrode area
- \( R_{XE} \) electrode area
ideal electrode: \( R_{XE} \rightarrow \infty \)

\[ \Delta V_{RE} : \]
depends on properties of electrolyte (e.g. ion concentrations) and electrode material

- "True" RE (e.g. Ag/AgCl): \( \Delta V_{RE} \) approx. const. independent of electrolyte properties
- "Quasi" RE (e.g. Au): suitable if electrolyte properties (concentrations) do not change too much during application


Potentiostatic Setup

Requirements

Stability:
- mandatory
- degrees of freedom to guarantee stability:
  - amplifier design
  - electrode areas (\( \rightarrow R_{XE}, C_{XE} \))
  - electrode arrangement on chip
    \( \rightarrow R_{XX-XX}, C_{XX-XX} \)

approach:
- determine open loop transfer function as a function of \( R_{XE}, C_{RE}, R_{XX-XX}, C_{XX-XX} \)
- roughly estimate \( R_{XE}, R_{XX-XX}, C_{XX-XX} \) as a function of electrode areas and spatial electrode arrangement
- choose advantageous electrode area and arrangement configuration and determine required operational amplifier properties (GBW, phase margin, ...)

Further required properties
- open loop gain: in many cases relaxed requirements
- offset voltage: in many cases relaxed requirements
- GBW / slew rate
  - detection method providing quasi DC output signal: low
  - detection method using transient signals: moderate ... high

On-Chip Potentiostatic Setup

Electrode Arrangements

Simplifications / boundary conditions:

- Electrolyte:
  Main contribution from resistive components, i.e. $C_{xx-xx}$ usually negligible for practical purposes

- Electrodes:
  - Main contribution from capacitive components, i.e. $R_{xe}$ usually negligible for practical purposes,
    since $R_{xe} \gg |j\omega C_{xe}|^{-1}$
  - $\Delta V_{xe}$: do not contribute to stability issues

... after some maths we derive ...

Electrode arrangement related design goals:

- Minimize $R_{RE-CE}$
- Make sure $R_{RE-CE}<R_{WE}$
- Make sure $C_{CE}>C_{WE}$
- Make sure $C_{RE}, C_{CE}>C_{in}$
  (more or less automatically fulfilled when using reasonable areas for electrodes and op-amp input MOSFETs)

Potentiostat Amplifier Circuits (Examples)

Example I:

- Potentiostatic setup for general purpose electroanalytical instrumentation
- Capability to drive moderate capacitive and large resistive loads
- Design for high precision purposes

Choice: Two-stage amplifier
(second input branch used for error + offset compensation purposes)

Example II:

- Sensor array with quasi DC signals (Redox-Cycling chip) → only low BW required
- DC output current drive capability

Choice: Simple folded cascode

$\mu A_{\text{gain}} = \frac{g_{m2}}{2 \pi C_{\text{LOAD}}}$

Design approach:

- Bias current output stage = $100 I_{\text{out,max}}$
- $I_{\text{out,max}}$ estimated on the basis of the known sensor properties and array size

Reliable design of entire on-chip potentiostatic setup can be achieved by careful electrode arrangement combined with suitable, relatively simple amplifier topology!
2. DNA and Protein Microarray Chips

2.4 Electrochemical Readout

2.4.3 Readout Circuits

Sensor Site Circuit with Charge Evaluation
Detection principle: Chronocoulometry
Basic approach: In-pixel current integration

Result:

\[ V_{\text{out}} = V_{\text{step}} + (n \times q \times D_{\text{probe}} + V_{\text{step}} \times C_{\text{int}} \times \alpha) \times \text{area} / C_{\text{int}} \]

Op-amps with slew rates of order 1V/µs required to distinguish electrode related signals (relatively fast) from background contributions (relatively slow).
Sensor Site Circuits with In-pixel Charge Evaluation (I)

Detection principle: Chronocoulometry

Approach:

- $10 \times 5$ array with $160 \mu m \times 120 \mu m$ pixel
- programmable multi-purpose detection pixel circuit
- fully differential pixel to suppress electrochemical fluctuations
- each pixel: two op-amps (gain-boosted folded cascades with switched biasing to lower $1/f$ noise), one differential amplifier, nine switches to select operation mode (slide shows configuration suitable for chronocoulometric measurements)

Roland Thewes, "Introduction to CMOS Bio Sensor Arrays: Electrical Specifications, CMOS Processing, Circuit and System Design", ISSCC 2009 Tutorial Short Course

Sensor Site Circuits with In-pixel Charge Evaluation (II)

Detection principle: Chronocoulometry

Approach:

- $24 \times 16 (=2 \times 12 \times 16)$ array, sensor site pitch $= 165 \mu m$
- two electrodes share one fully differential op-amp
- the signal from each electrode can be evaluated independently due to input common-mode feedback concept

Roland Thewes, "Introduction to CMOS Bio Sensor Arrays: Electrical Specifications, CMOS Processing, Circuit and System Design", ISSCC 2009 Tutorial Short Course
Sensor Site Circuit with In-Site Current A/D Conversion

Detection principle: Redox-Cycling

Requirement: Precise measurement of quasi-DC current

- simultaneous data sampling from all sensor site independent of the number of test sites per array
- excellent robustness / signal integrity

process: 3M 2P 0.5µm 5V CMOS, extended by Au electrodes

M. Schienle et al., JSSC, 2004

Entire Redox-Cycling Chip*

Requirement:
Precise measurement of quasi-DC current

serial interface circuitry
row & column selection
2x2 DACs
bandgap circuit & generation of reference currents

Electrochemical test: excellent performance over > 3.5 decades (for comparison: state-of-the-art optical systems provide approximately 2.5 – 3 decades)

16x8 sensor array with in sensor-site A/D conversion, pitch = 250 µm

* since May 05 property of Siemens Medical Solutions

A. Frey et al., ISCAS 2005

Roland Thewes, "Introduction to CMOS Bio Sensor Arrays, Electrical Specifications, CMOS Processors, Circuit and System Design", ISSCC 2008 Tutorial Short Course
2. DNA and Protein Microarray Chips

2.5 Label-Free Approaches

2.5.1 Quasi Labeling-Free Electrochemical Approaches

Quasi Labeling-Free Electrochemical Approaches
Definition and Examples

Definition:
"Quasi labeling-free" shall mean here, that the labeling step applied to the target molecule is avoided. It shall not mean, that the use of label molecules in the entire assay is generally circumvented.

The following examples are applicable on similar / same chips as introduced before to operate labeling-based electrochemical detection assays.

Examples:

Electrochemical displacement assays
- Use of labeled "signal oligos"
- Targets displace signal oligos in case of (perfect) match
- Performance of the method depends on oligo design
- Promising approach to achieve high specificity!

signal oligo

label molecule

match

mismatch
Quasi Labeling-Free Electrochemical Approaches
Examples cont'd

Intercalators
- Intercalators are captured in between double-stranded DNA molecules during the hybridization process
- Intercalators carry label molecules
- Method allows to achieve relative high numbers of labels associated with a matching DNA double-strand

Intercalator

label molecule

G-oxidation within target strand
- Guanine in probe strands must be replaced by Inosine
- Signal depends on the amount of G in target strand
- Method destructive
- Oxidation voltage of other bases close to that of G (of order 1V)
- Reliability? Repeatability?

2. DNA and Protein Microarray Chips

2.5 Label-Free Approaches

2.5.2 Impedance-Based Sensors
Label-Free Approaches: Impedance Method

**Basic principle**

- C: parameter of interest
- R: artifact dependent
- Phase sensitive characterization required to detect the biological information

(Literature: Different electrode arrangements / layouts in use)

- C, R, ΔC, ΔR depend on the quality of the layer of probe molecules (method is sensitive to pinholes in that layer)
- Literature reveals a number of proof-of-principles, but a consistent picture has not yet been achieved (data prone to measurement artifacts?)
- Active CMOS has the potential to avoid measurement artifacts due to signal processing close to the sensor and may thus help to evaluate the method

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**Example: Simple SC Setup to Distinguish R and C Contribution**

**Principle:**

\[ I = f C V + T_{on} f V / R \]

**Average current:**

- **Au-oligo**
- **Au-noncomplementary.oligo**
- **Au-complementary.oligo**
- **Au-oligo.reset**
- **Au-pBR322**

Proof-of-principle performed using discrete electronic devices

C. Guiducci et al., ESSDERC 2002

**Graph:**

- **Frequency (Hz)**: 0 to 100
- **Average Current (μA)**: 0 to 250

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Seite 25
Label-Free Approaches: Impedance Method

Example: Oscillator Circuit Setup to Determine C (and R)

Approach:

- $16 \times 8$ array, sensor site pitch = $300\mu m$
- each pixel: dual-slope impedance-to-frequency-based ADC
- Evaluation of frequency as a function of bias current $I_{\text{ref}}$ reveals $C$ and $R$:
  \[
  \frac{1}{f} = C \times R \times \ln \left\{ \frac{1}{1 - \frac{V_{\text{ref}}}{I_{\text{ref}} \times R}} \right\}
  \]
- Approximation for not too low $I_{\text{ref}}$:
  \[
  C = \frac{I_{\text{ref}}}{2 \times V_{\text{ref}} \times f}
  \]

C. Stagni et al., ISSCC 2006

2. DNA and Protein Microarray Chips

2.5 Label-Free Approaches

2.5.3 Gravimetric Sensors
Label-Free Approaches
Basic Principle of Gravimetric Sensors

- Mass sensitive sensors (as considered here) are mechanical / electrical oscillating systems.
- They are described by the basic oscillation equations in the electrical and in the mechanical domain.

\[
LQ'' + RQ' + \frac{Q}{C} = U_a \cos(\omega t) \\
mx'' + kx' + Dx = F \cos(\omega t)
\]

- Mass and viscosity changes due to biological binding events at the sensor surface change the oscillation frequency:

**mass-related effect:** \( \Delta \omega / \omega \approx \Delta m / m \)

**viscosity-related effect:** \( \Delta \omega / \omega \approx \Delta Q / Q \) (here: \( Q \) = quality factor!)

Label-Free Approaches
Properties of Gravimetric Sensors

- Equivalent circuits of related sensors usually consist of more lumped elements as given in the basic equations on former slide.
- Mass sensitive sensor methods are tolerant against pinholes in the receptor molecule layer.
- If operated in water, strong damping occurs. As a consequence, the quality factor significantly degrades. Active circuitry and operation of the sensor in closed loop configuration is a must to achieve sufficient system performance.

**Example:** Cantilever (cf. next slide) operated in different environments and configurations.

Y. Li et al., IEEE Sensors 2003

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**Seite 27**
Mass-Sensitive Label-Free Sensors
Monolithically Integrated Cantilever on CMOS chip (I)

CMOS post-processing flow to fabricate cantilever:

- Removal of silicon n-well (cantilever release)
- Removal of silicon dioxide dielectric layer
- Removal during chip separation ("dice line")
- Magnetic field by permanent magnet
- Etch at silicon n-well
- Dielectric layers
- Bulk silicon

Cantilever operated in resonance mode:

- Wheatstone bridge
- Lorentz force
- Current loop

Mass-Sensitive Label-Free Sensors
Monolithically Integrated Cantilever on CMOS chip (II)

Architecture:

- Wheatstone bridge
- LNA
- HP-filter
- VGA
- Digital output
- Amplitude limiter
- AP-filter
- Analog feedback loop

Chip photo:

- 4 resonant cantilevers
- 2M 2P 0.8\mu m CMOS
Mass-Sensitive Label-Free Sensors
Film Bulk Acoustic Wave (BAW) Sensor (I)

Schematic cross section:

Equivalent circuit:

- Suitable materials (e.g. AlN) in principle compatible with CMOS processes and already in use for other MEMS applications.
- Operating frequencies in the low GHz range.

G. Sauerbrey, Zeitschrift für Physik 155, 1959

Mass-Sensitive Label-Free Sensors
Film Bulk Acoustic Wave (BAW) Sensor (II)

Frequency [GHz]

Measured shift of resonance frequency

- Sensor mounted on PCB and operated with oscillator circuit consisting of discrete components here.
- CMOS integration: on-going.

R. Brefertow et al., IEDM 2003
2. DNA and Protein Microarray Chips

2.6 Packaging Aspects

Packaging aspects

- Packaged electronic biochips require a fluidic and an electric interface. Interfacing effort in case of optical biochips (fluidic + optical interface) is not higher!
- Electronic biochips: Cheap and reliable packaging solution required.
- Requirements concerning in-package (micro-) fluidics:
  - laminar flow
  - bubbles must be avoided (or trapped at predefined positions within package)
  - detailed requirement catalogue depends on detection method / assay / application

**Insufficient packaging / micro-fluidic solutions may significantly deteriorate the performance of the entire system.**

Example:

DNA experiment measured on wafer-level using a simple flow cell. Circuit accuracy $\sigma < 1\%$
Packaging Examples

Nanogen  Toshiba  ETH Zurich  Combimatrix  Siemens  (under development)

3. Cell-based Sensor and Actuator Chips

3.1 Cell Manipulation and Cell Sorting
CMOS Chips for Cell Manipulation and Cell Sorting
Use of Dielectrophoretic Cages (I)

Goal: Highly parallel, individual, non-invasive cell manipulation

Applications:
- cell counting (→ blood analyses)
- individual cell isolation (→ biopsies)
- cell-to-cell interactions (→ immune response studies)
- compound delivery into cells (→ drug development)
- tissue assembly
- ...

Approach: CMOS chip to
- control AC voltages applied to electrodes (isolated from electrolyte), which generate together with a conductive lid of the microchamber - the required dielectrophoretic forces
- monitor the positions of the cells using photodiodes or capacitive sensing

CMOS Chips for Cell Manipulation and Cell Sorting
Use of Dielectrophoretic Cages (II)

Setup: chip on PCB
- bonding wires
- protection
- conductive grease

A. Romani, ISSCC 2004

<table>
<thead>
<tr>
<th>chip area</th>
<th>64mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>array size</td>
<td>320 x 320</td>
</tr>
<tr>
<td>pitch</td>
<td>20μm</td>
</tr>
<tr>
<td>microchamber</td>
<td></td>
</tr>
<tr>
<td>- height</td>
<td>85...100μm</td>
</tr>
<tr>
<td>- volume</td>
<td>&lt;3.5...5μm</td>
</tr>
<tr>
<td># of DEP cages</td>
<td>up to ~1000</td>
</tr>
<tr>
<td>VDD</td>
<td>3.3V</td>
</tr>
<tr>
<td>actuation</td>
<td></td>
</tr>
<tr>
<td>- voltages</td>
<td>3.3V (ext. applied)</td>
</tr>
<tr>
<td>- frequency</td>
<td>100kHz – 10MHz</td>
</tr>
<tr>
<td>Clk frequency</td>
<td>20MHz</td>
</tr>
</tbody>
</table>

Summary specifications (opt. and cap. sensing chip)

CMOS Chips for Cell Manipulation and Cell Sorting

Alternative Approach: Use of Magnetic Forces

Approach:
- Cells labeled with magnetic beads:

  ![Magnetic bead diagram]

- Chip: array with selectable coils to magnetically move and trap cells
- Current per coil: ≥10mA
  - larger arrays require current multiplexing
  - magnetic force experienced by cells: 20...50pN

Prototype:
- 3M SiGe Bipolar Chip, 5x5 array, pitch = 25μm
- next generation: 0.18μm CMOS, 8x8 array, pitch = 25μm

3. Cell-based Sensor and Actuator Chips

3.2 Nerve Cell and Neural Tissue Interfacing
Nerve Cell and Neural Tissue Interfacing

Nerve Cells

Goal:
Measurement of action potentials

Action potentials:
- are elementary neural signals
- are transient changes of the transmembrane voltage
- correspond to sodium and potassium ion currents through ion channels in the cell membrane.

Further remarks:
- typical cell diameters: 10...100μm
- steady-state potential of the transmembrane voltage also depends on amount of further ions such as Cl, Ca**, ..., and on further mechanisms.

Patch-Clamping

Setup

- Direct contact to intracellular space
- Gold standard in electrophysiology
- Used to characterize gating characteristics of ion channels
- Different patch techniques in use
- Different configurations in use

- Low throughput
- Time expensive
- Trained staff required
- Stable mechanical support obligatory
- Not capable for multi-site recording

Roland Thewes, "Introduction to CMOS Bio Sensors: Electrical Specifications, CMOS Processing, Circuit and System Design", ISSCC 2006 Tutorial Short Course Page 34
Nerve Cell and Neural Tissue Interfacing
Extracellular Non-Invasive Recording

Principle of extracellular recording:
Ion currents flowing through cleft between cell and surface of solid state substrate lead to transient changes of cleft voltage with respect to electrolyte bulk potential.

Cleft voltage monitoring techniques:
- Metal electrode (e.g. Au, Pt, ...)
  - on-chip or off-chip amplifier
- Noble metal electrode-to-electrolyte contact:
  - contact via Helmholtz layer
  - non-homogeneous surface

Electrolyte-Oxide-Semiconductor-FET (EOSFET):
- cleft voltage modulates OSFET current
- homogeneous (dielectric) surface

Typical peak-to-peak cleft voltages: 100μV - 5mV

Nerve Cell and Neural Tissue Interfacing
Non-CMOS Approaches

Passive Multi Electrode Arrays (MEAs) with Metal Electrodes:
- no active electronic devices on chip
- commercially available
- transparent substrates
- simultaneous sensing and stimulation
- approximately 60 test sites per array
- pitch of order 200μm
- further increase of # of sites / of site density limited by interconnect restrictions

EOSFETs:
- many proof-of-principles using metal-free processes (i.e. entire wiring in diffusion layer)
- simultaneous sensing and stimulation demonstrated
- 1D pitch of order few μm
- further increase of # of sites / of site density in 2D arrangements limited by interconnect restrictions
Nerve Cell and Neural Tissue Interfacing
CMOS MEA with Metal Electrodes

- 16x8 electrodes, pitch: 250μm
- total chip area: approx. 42mm²
- 3M 2P 5V 0.6μm CMOS process, 2 mask postprocessing
- each site with integrated bandpass filtering and signal buffering
- simultaneous sensing and stimulation
- fully digital chip interface, system interface with USB 2.0

F. Herr et al., ESSCIRC 2005

Nerve Cell and Neural Tissue Interfacing
High-Density 2D Imaging

- Goal:
  Pitch of order 10μm or below, total sensor area 1mm²
- Approach:
  Extended EOSFET sensing
- Challenges:
  1. Sensor dielectric
     - high k / low thickness (εr = 20...80, t = 50 nm)
     - non-toxic, biocompatible
     - CMOS compatible
     - leak-proof
     - processed at T ≤ 400° C
  2. Floating node realization impossible: operating point of sensor transistor prone to large uncontrollable processing-induced charging effects!
  3. Fixed pattern noise (FPN) resulting from sensor transistor σ(Vt) >> signals to be detected

High-Density 2D Neural Tissue Imaging CMOS Chip
Design Approach

Sensor pixels: sensor pixels

Periodically repeated calibration of pixels to cancel FPN:

Calibration:

\[ I \propto (V_{Gk} - V_{th})^2 \]

= const.

Readout:

\[ \Delta I \propto g_m \Delta V_{Gk} \]

= const

Entire signal path:

Readout circuitry

On-Chip/Off-Chip

CMOS Imager Chip for Extracellular Monitoring of Neural Tissue with 16k Pixels on 1mm²

Cultivated snail cell on chip surface

Sensor size 128 x 128
Sensor area 1mm x 1mm
Sensor pitch 7.8μm
Chip size 5.4mm x 6.5mm
Process 2P 3M 0.5μm CMOS
Frame rate > 2000 Fps
Data rate 32MS/s
Power cons. 656mW
VDD 5 V

Packaged chip

Sensor size 128 x 128
Sensor area 1mm x 1mm
Sensor pitch 7.8μm
Chip size 5.4mm x 6.5mm
Process 2P 3M 0.5μm CMOS
Frame rate > 2000 Fps
Data rate 32MS/s
Power cons. 656mW
VDD 5 V
CMOS Imager Chip with 16k Pixels on 1mm²
Mapping of Neural Network Activity


4. Further CMOS-/Si-Related Applications
Further Applications
Multi Parameter Metabolism Sensing Chips
Example:
Blood gas sensor for continuous monitoring of vital metabolic parameters (pH, pO₂, pCO₂)

Setup approach:

Ion-sensitive FET (ISFET) based sensor approach:
- micropool for membrane dispensing
- passivation
- micropool for electrolyte dispensing
- polyimide
- junction poly
- ILD thermal nitride
- silver platinum metal
- MOSFET
- ISFET

Flow-through cell with integrated sensor for pO₂, pCO₂, pH, temperature, ... and heater (37 °C)

- Pump modules
- flushing solutions
- Electronic control: Laptop

Chip photo:
- extended 1.2μm
- CMOS process

E. Lauwers et al., ISSCC 2001

Further Applications
Example: MEMS Chip for DNA Sample Preparation

MEMS PCR* Chip

- Denaturation (T = 95 °C)
- Add Primers
- Double stranded DNA
- Extension (T = 50...72 °C)
- AGCTTACG
- AGCTTACG
- TCGAATGCC
- AGCTTACG
- TCGAATGCC
- AGCTTACG

* Polymerase Chain Reaction (Schematic flow chart)

B. Vigna, Chips-to-Hits 2002
Further Related Approaches
Prosthetic Devices

Cochlear Implants

• restoring hearing to the profoundly deaf
• 100,000 prostheses worldwide in use
• development further on-going

Retinal Implants

• goal: restoring seeing
• chip measures light signals and stimulates nerves
• status of development lower as compared to cochlear implants
• a number of promising projects worldwide under development

K. Wise, IEDM 2002

5. Summary
Summary

CMOS-based biosensor arrays have been discussed considering
- extended CMOS processing issues
- various sensor techniques and related electrical characteristics
- circuit design approaches to fulfill related requirements
- system aspects.

Status today:
- Many technical challenges related to the development of
  CMOS-based biosensors can be overcome.
- A number of demonstrators have proven that extended CMOS
  chips are able to open novel and user-friendly bio sensor/actuator
  solutions.

But:
A "solution" must always consider the entire system/application
chain. (i.e., that goes beyond considering the CMOS chip alone!)
- Commercialization has not yet widely been achieved, but is on-going.

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