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CMOS DNA Microarrays: Circuit and System Aspects

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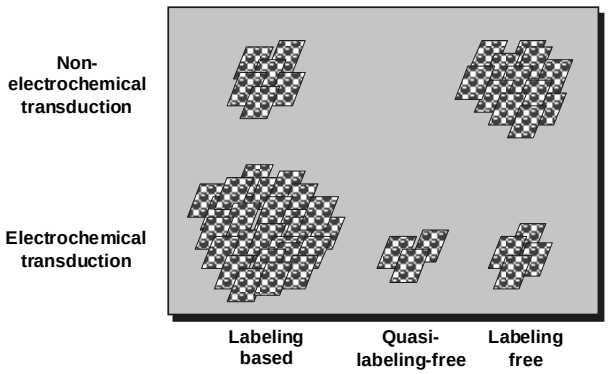
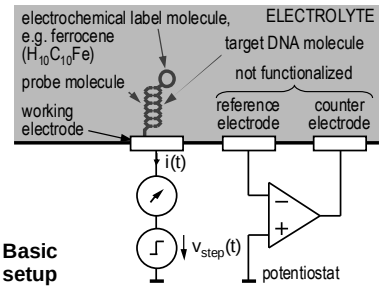
Outline (Part II)
CMOS DNA Microarrays: Circuit and System Aspects

Overview
Classes of Electronic Bio Molecule Detection Techniques

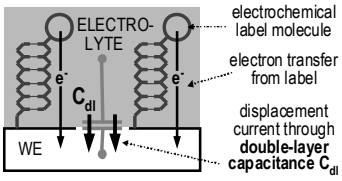


Estimated relative amount of chip- or CMOS-related publications.

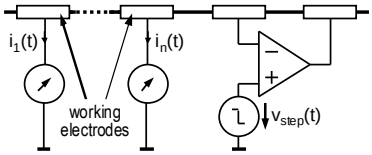
Coulometric Readout
Three-Electrode-System



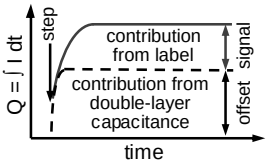
Basic setup



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



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



Result (schematic plot)

Coulometric Readout
Signal-to-Offset-Ratio

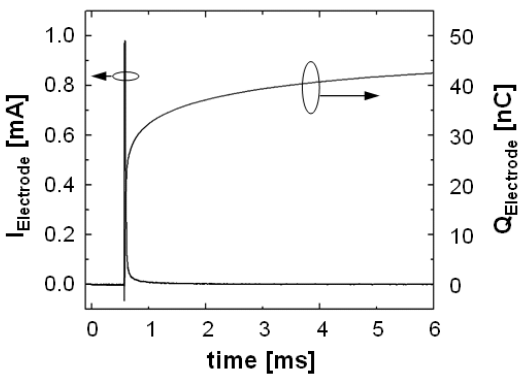
$$\text{Signal-to-Offset-Ratio } \mathbf{SOR} = \frac{\text{charge from label molecules}}{\text{charge from double-layer capacitance}} = \frac{n \times q \times D_{probe}}{V_{step} \times C_{dl}}$$

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

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

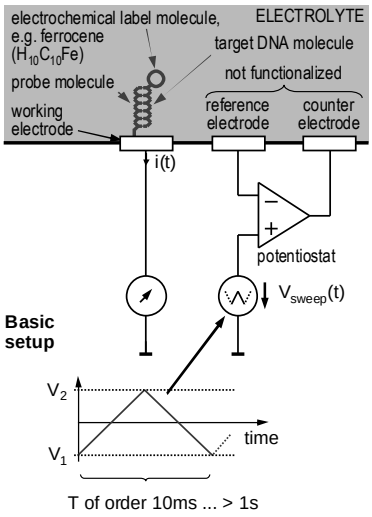
Requirements / guidelines for practical applicability:
- Decrease C_{dl}:
  → Introduce electrode surface blocking layer after probe molecule immobilization
- Increase n (by using other / modified label molecules)
- Increase # label molecules per target
- Use labels with decreased values of V_{step}

Coulometric Readout
Typical Signals



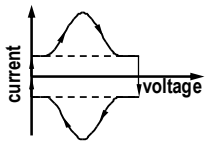
Example from large area electrode experiment
Signal magnitude: Q_{total} / area ~ of order 10 nC / mm²

Cyclic Voltammetry
Three-Electrode-System

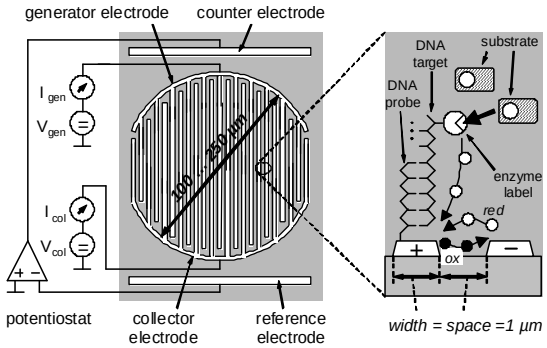


- Voltage is swept from V_1 to V_2 and back, so that a complete redox-cycle is performed.
- Working electrode current is measured and
  - signal peak current
  - signal peak-to-peak current
  - area in between curves (∝ total charge) is evaluated.
- Note, that current depends on slew rate of potentiostat input voltage

Ideal Cyclic Voltammetry I-V diagram:

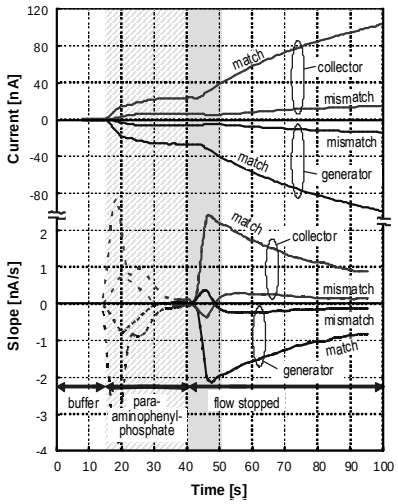


Redox-Cycling
Four-Electrode-System with Interdigitated Working Electrodes



- 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 which 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
Typical Signals



- Measurement time:
  seconds
- Frequently evaluated signal:
  ∂ current / ∂ time

  Motivation:
  absolute current value may also contain contributions of time-independent artifacts ("electrochemical x-talk")
- Required resolution:
  1 pA ... 100 nA

  Under assumptions:
  - sensor diameter of order 200 µm
  - electrode width and spacing ~ 1 µm
  - suitable for wide range of applications

Quasi Labeling-Free Electrochem. Approaches
Definition and Examples

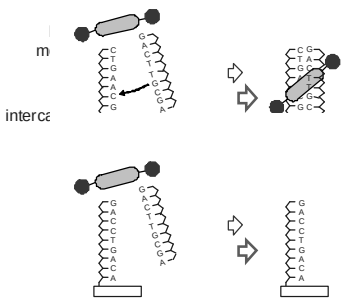
Definition:

"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 using similar / same electrochemical configurations for detection as introduced before to operate labeling-based electrochemical detection assays.

Quasi-Labeling-Free Electrochem. Approaches
Example: Intercalators



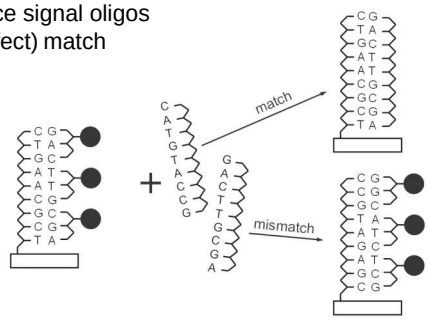
- Intercalators are captured in between double-stranded DNA molecules during the hybridization process
- Intercalators carry label molecules
- The presence of label molecules can be detected using one of the electrochemical measurement setups shown before
- Method allows to achieve relative high numbers of labels associated with a matching DNA double-strand

- Problem: toxicity

e.g. N. Gemma et al., ISSCC 2006
http://dna-chip.toshiba.co.jp/eng/

Quasi Labeling-Free Electrochem. Approaches
Example: Displacement Assays

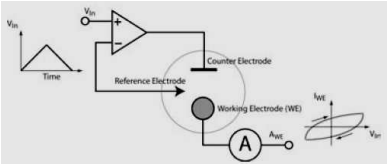
- Use of labeled "signal oligos"
- Targets need not to be labeled
- Targets displace signal oligos in case of (perfect) match

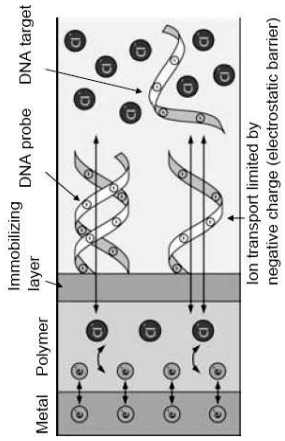


Labeling-Free Electrochemical Readout
Example: Electropolymer Redox Reaction

Principle:

- Cyclic Voltammetry (3 electrode system) applied to oxidize and reduce an electropolymer (polypyrole) covering the working electrode
- Hybridization hinders movement of the chloride counter ions and thus decreases the measured redox currents and related shapes of the cyclic voltammetry curves





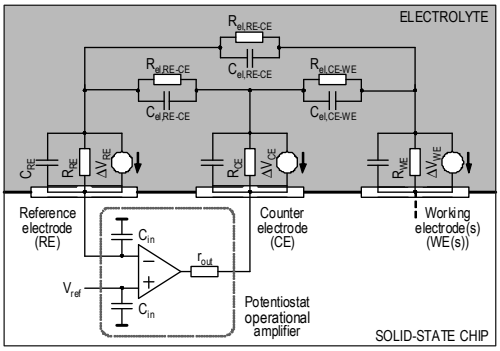
F. Heer et al., ISSCC 2008

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

Potentiostatic Setup

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

Setup / Configuration:



Parameters:

$R_{el,XX-XX}$, $C_{el,XX-XX}$:
depend on
- electrolyte properties (concentration, compounds, ...)
- distances between respective electrodes

R_{XE}, C_{XE} :
depend on
- properties of electrolyte and electrode material
- $C_{XE} \propto$ electrode area
- $R_{XE} \propto 1$ / electrode area
  ideal electrode: $R_{XE} \rightarrow \infty$

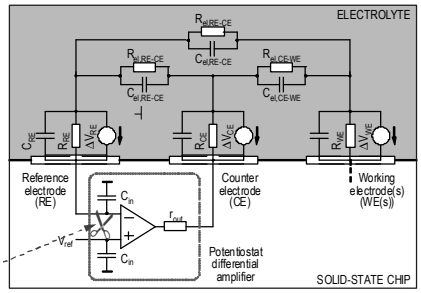
ΔV_{XE} :
depends on properties of electrolyte (e.g. ion concentrations) and electrode material

- "True" RE (e.g. Ag/AgCl): ΔV_{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

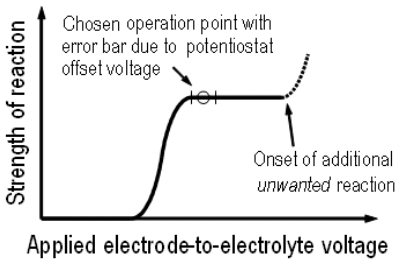
Stability:

- mandatory
- degrees of freedom to guarantee stability:
  - amplifier design
  - electrode areas (→ R_{XE}, C_{XE})
  - electrode arrangement on chip (→ $R_{el,XX\text{-}XX}$, $C_{el,XX\text{-}XX}$)
- approach:
  - determine open loop transfer function as a function of R_{XE}, C_{XE}, $R_{el,XX\text{-}XX}$, $C_{el,XX\text{-}XX}$
  - roughly estimate R_{XE}, C_{XE}, $R_{el,XX\text{-}XX}$, $C_{el,XX\text{-}XX}$ 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, ...)



Potentiostatic Setup
Further Requirements

- open loop gain: in many cases relaxed requirements
- offset voltage: in many cases relaxed requirements



- GBW / slew rate
  - detection methods providing quasi DC output signals: low
  - detection method using transient signals: moderate ... high

On-Chip Potentiostatic Setup
Electrode Arrangements To Achieve Stability

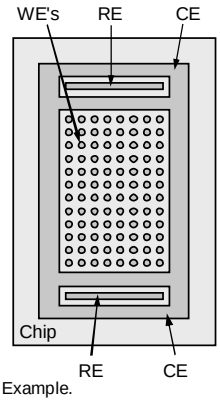
Simplifications / boundary conditions:

- **Electrolyte:**
  Main contribution from resistive components,
  i.e. $C_{el,XX\text{-}XX}$ usually negligible for practical purposes
- **Electrodes:**
  - Main contribution from capacitive components, i.e. R_{XE} usually negligible for practical purposes, since $R_{XE} >> |j\omega C_{XE}|^{-1}$
  - ΔV_{XE}s: do not contribute to stability issues

... after some maths we derive ...

Electrode arrangement related design goals:

- Minimize $R_{el,RE\text{-}CE}$
- Make sure $R_{el,RE\text{-}CE} << R_{el,RE\text{-}WE}$
- 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)



Example.

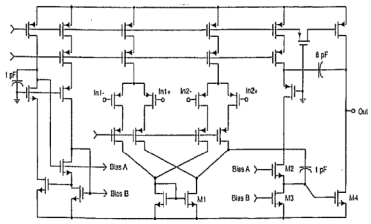
Further arrangement aspects may depend on the specific assay used.

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)



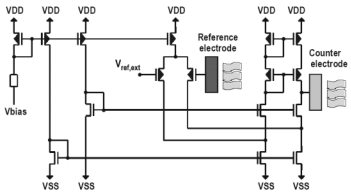
2µm CMOS **R. Reay et al., ISSCC 1994**

Example II:

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

Choice: Simple folded cascode

- $f_{unity\,gain} = g_{m,IN} / 2\,\pi\, C_{LOAD}$
- design approach:
  bias current output stage = 100 $I_{OUT,max}$
  ($I_{OUT,max}$ estimated on the basis of the known sensor properties and array size)

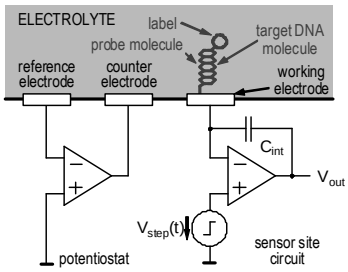


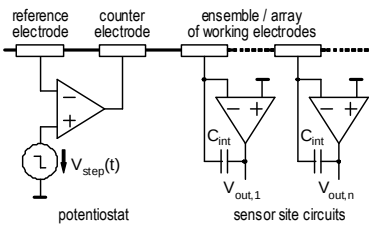
3M 2P 0.5µm 5 V CMOS **A. Frey et al., ISCAS 2003**

Reliable design of entire on-chip potentiostatic setup can be achieved by careful electrode arrangement combined with suitable, relatively simple amplifier topology!

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

Pixel Circuitry for Coulometric Detection
Basic Approach: In-Pixel Current Integration





Result:
V_{out} =
$V_{step} + (n \times q \times D_{probe} + V_{step} \times C_{dl} \times \alpha) \times \text{area} / C_{int}$

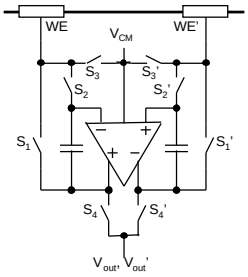
Result:
V_{out} =
$(n \times q \times D_{probe} + V_{step} \times C_{dl} \times \alpha) \times \text{area} / C_{int}$

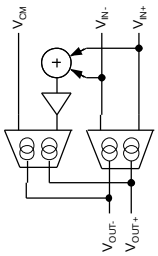
Op-amps with slew rates of order 1V / µs required to distinguish electrode related signals (relatively fast) from background contributions (relatively slow).

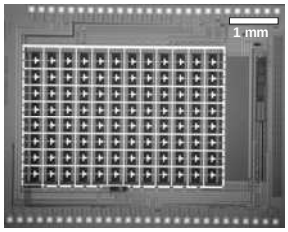
Pixel Circuitry for Coulometric Detection
Example: Pixel Topology and Integrator

Approach:

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



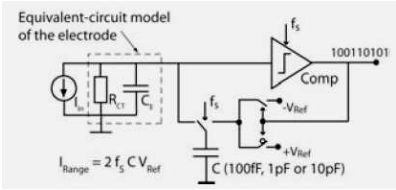


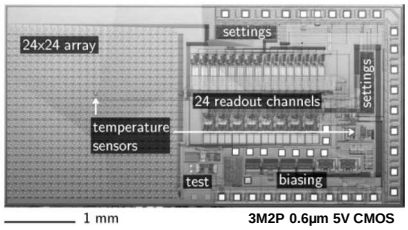


hardware realization in 3M 2P 0.5µm 5V CMOS
M. Augustyniak et al., ISSCC 2006

Pixel Circuitry for Cyclic Voltammetry
Example: 1st Order Delta-Sigma ADC

- 24×24 array with 24 channels
- Pt electrode sensor sites, diameters from 10 µm to 40 µm, pitch = 100 µm
- Detection method: cyclic voltammetry
- Each channel of the array equipped with 1st order delta-sigma ADC:
  - sensor site electrode used as integration capacitance
  - very high OSR due to very slow nature of electrochemical signals (in this case!)
  - achieved ENOB ≈ 11

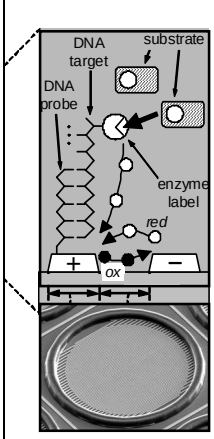


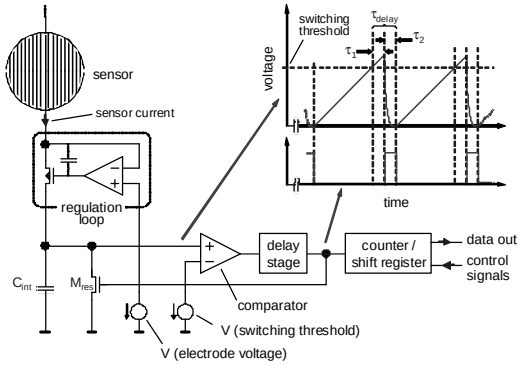


3M2P 0.6µm 5V CMOS

F. Heer et al., ISSCC 2008

Pixel Circuitry for Redox-Cycling
Example: Single Slope ADC within Each Sensor Site

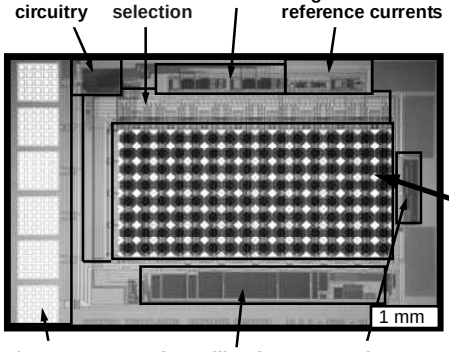




- simultaneous data sampling from all sensor sites independent of the number of test sites per array
- excellent robustness / signal integrity
- process: 3M 2P 0.5µm 5V CMOS, extended by Au electrodes

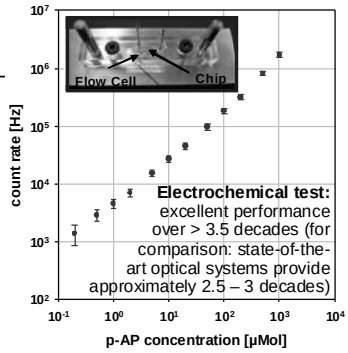
M. Schienle et al., JSSC, 2004

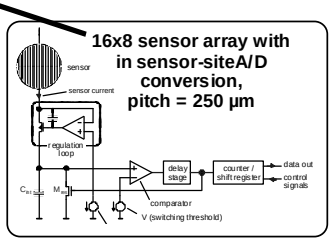
Example:
Entire Redox-Cycling Chip*



3M 2P 0.5 µm 5 V CMOS

A. Frey et al., ISCAS 2005





* since May 05 property of Siemens Medical Solutions

Outline (Part II)
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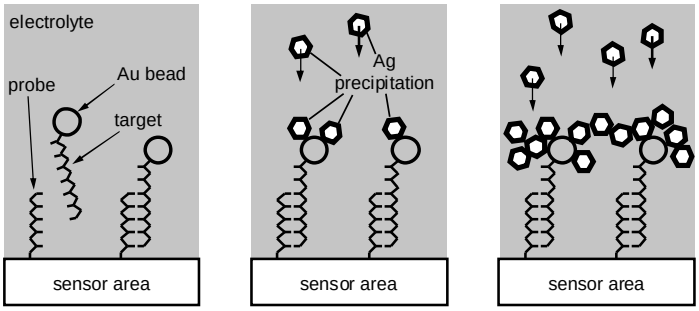
Non-Electrochemical Labeling-Based Transducer Techniques

Approach: Gold Bead Labeling + Silver Precipitation. Basic Principle:

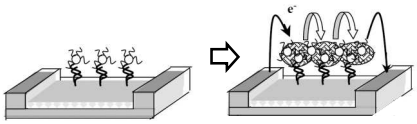


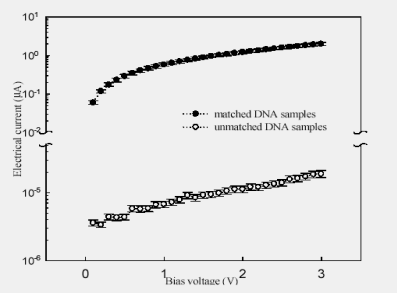
Hybridization-to-electrical signal transduction:

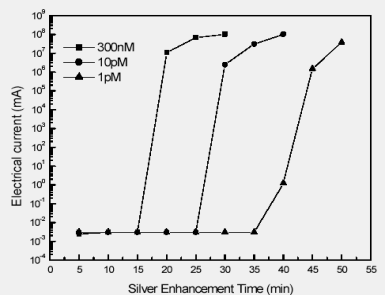
- Conductivity measurement between electrodes separated by isolating layer
- Impedance (or RF parameter) measurement between isolated electrodes
- Optical attenuation

Au Bead Labeling + Ag Precipitation
Conductivity Readout

- **Probe molecules are immobilized on isolating layer between electrodes**
- **Conductive Ag layer leads to a sharp decrease of ohmic resistance between the electrodes**
- **Discrimination "match" / "mismatch" positions requires consideration of temporal development**



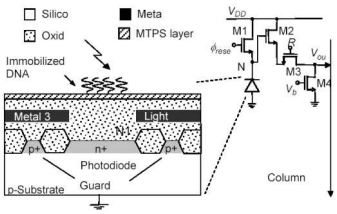


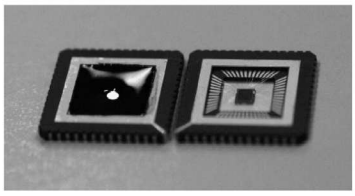


M. Xue et al., IEDM, 2002, and ISSCC, 2003

Au Bead Labeling + Ag Precipitation
Detection of Optical Attenuation

- **Approach 1:**
  Use of a (disposable?) CMOS camera chip (published by same authors who had used conductivity readout in former publications, cf. former slide)



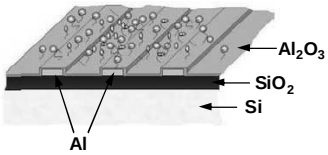


J. Li et al., IEDM 2004

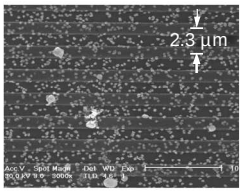
- **Approach 2:**
  Camera and entire optical system is part of the (non-disposable) readout apparatus, chip is a pure optical device without any integrated electronics

Au Bead Labeling + Ag Precipitation
Impedance / RF Parameter Readout

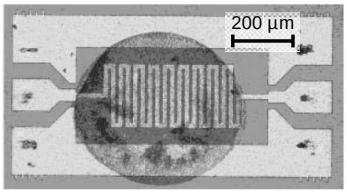
- **Probe molecules are immobilized on an isolating layer above capacitors with interdigitated electrodes, coil structures, meanders, ...**
- **Conductive Ag layer leads to a change of impedance / RF parameters of the electrical device**
- **Discrimination " match" / "mismatch" requires consideration of temporal development**



Schematic cross-section



Electrodes covered by silver grains after biological process



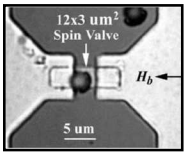
Spotted meander inductor structure

G. Laurent et al., ESSDERC 2003
L. Moreno-Hagelsieb, ESSDERC 2006

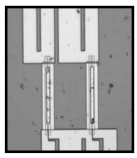
Non-Electrochemical Labeling-Based Transducer Techniques

Approach: Magnetic Bead Labeling. Basic Principle:

- 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), recently CMOS integration
- R&D further on-going

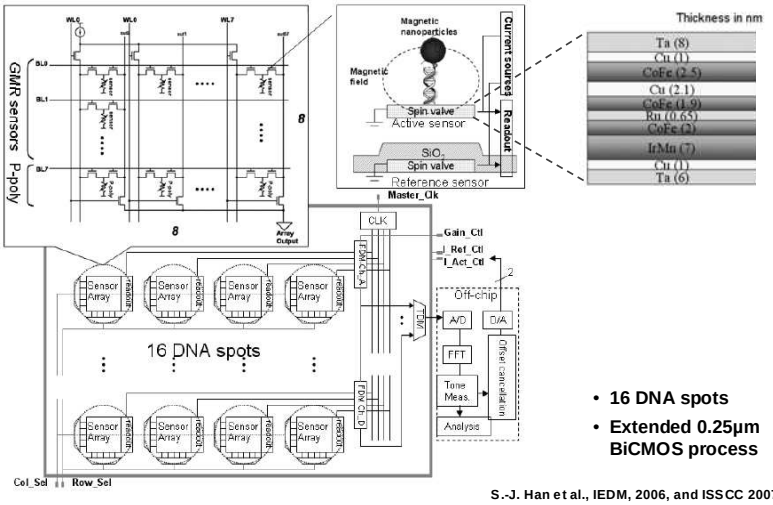


G. Li et al., J. Appl. Physics, 2003



M. Megens and M. Prins, J. Magnetism and Magnetic Materials, 2005

Magnetic Bead Labeling
Demonstration of CMOS Integration



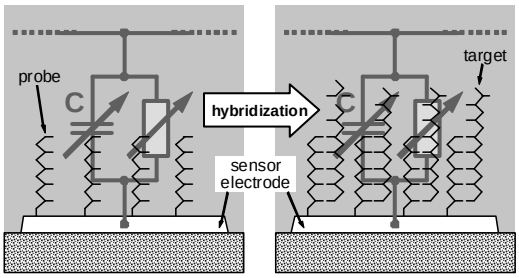
- **16 DNA spots**
- **Extended 0.25µm BiCMOS process**

S.-J. Han et al., IEDM, 2006, and ISSCC 2007

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

Non-Electrochemical Labeling-Free Transducer Techniques

Impedance Method



(Literature: Different electrode arrangements / layouts in use)

Basic principle

C: parameter of interest

R: artifact dependent

⇩

Phase sensitive characterization required to detect the biological information

- 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 completely consistent picture has not yet been achieved.

Non-Electrochemical Labeling-Free Transducer Techniques

Gravimetric Approaches

- 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.

$$LQ'' + RQ' + Q/C = U_a \cos(\omega t)$$

$$mx'' + kx' + Dx = F \cos(\omega t)$$

Q	charge	**t**	time
L	inductivity	**x**	position
R	resistance	**m**	mass
C	capacity	**k**	mechanical attenuation
$\mathbf{U_a}$	voltage amplitude	**D**	spring constant
ω	frequency	**F**	mechanical force

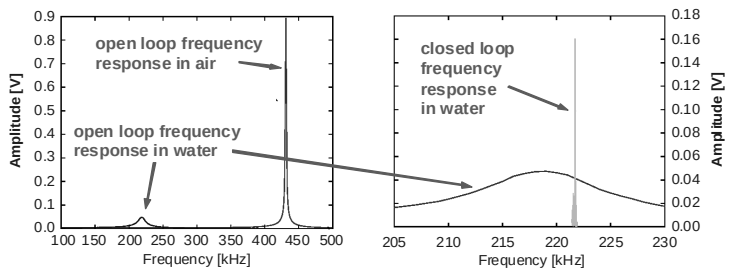
- Mass and viscosity changes due to biological binding events at the sensor surface change the oscillation frequency:

<u>mass-related effect:</u> $\Delta\omega / \omega \propto \Delta m / m$

<u>viscosity-related effect:</u> $\Delta\omega / \omega \propto \Delta Q / Q$ (here: Q = quality factor!)

Gravimetric Sensors
Properties

- 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.

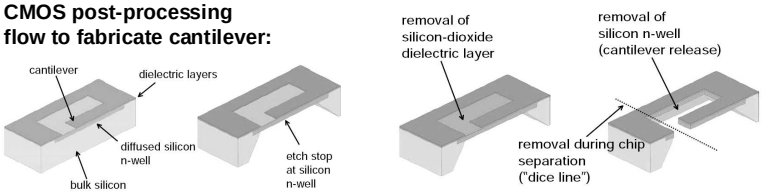


Example: Cantilever (cf. next slide) operated in different environments and configurations.

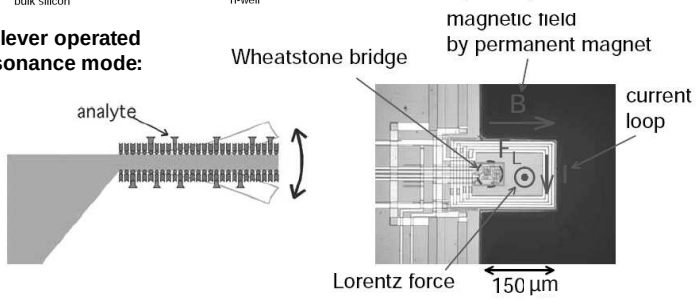
Y. Li et al., IEEE Sensors 2003

Gravimetric Sensors
Monolithically Integrated Cantilever on CMOS Chip (I)

CMOS post-processing flow to fabricate cantilever:



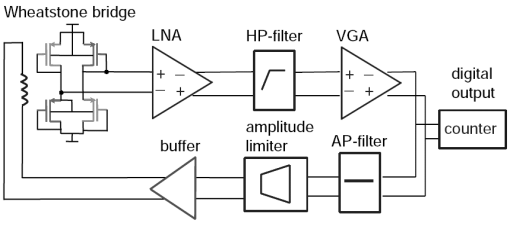
Cantilever operated in resonance mode:



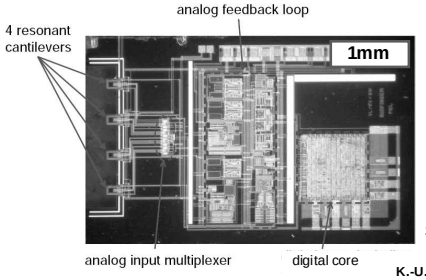
K.-U. Kirstein et al., DATE 2005

Gravimetric Sensors
Monolithically Integrated Cantilever on CMOS Chip (II)

Architecture:



Chip photo:

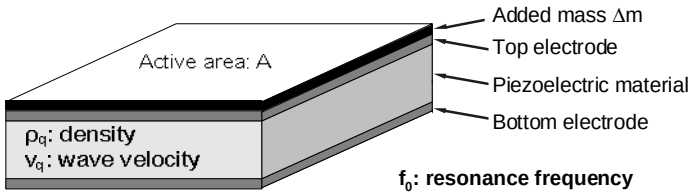


2M 2P 0.8µm CMOS

K.-U. Kirstein et al., DATE 2005

Gravimetric Sensors
Film Bulk Acoustic Wave Resonator (FBAR) (I)

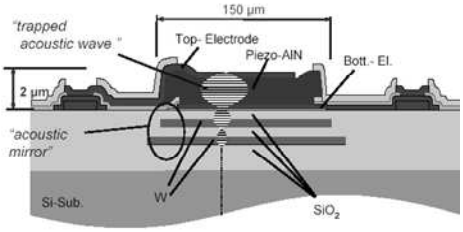
- Mass changes at the sensor surface change the oscillation frequency.
- Basic principle:



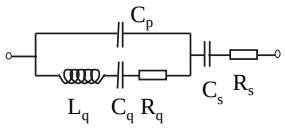
- Change of resonance frequency in air (Sauerbrey equation): $\frac{\Delta f}{f} = -\frac{2f_0}{A\rho_q v_q} \times \Delta m$
  - → high sensitivity requires high f_o
  - → thin-film piezoelectric layers resonating in the GHz range are superior compared to conventional quartz-based sensors operating in the MHz...tens of MHz range
- Mechanical attenuation:
  quality factor in liquids (water) is significantly lower as in gases (air) **!**

Gravimetric Sensors
Film Bulk Acoustic Wave Resonator (FBAR) (II)

Schematic cross section:



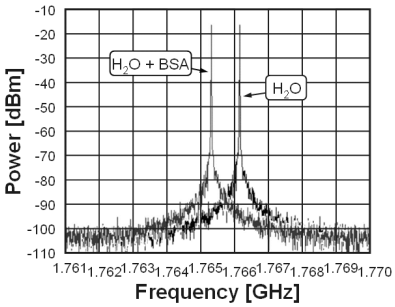
Equivalent circuit:



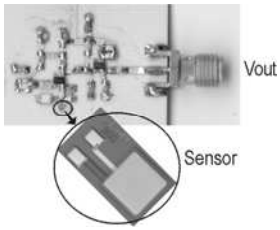
G. Sauerbrey, Zeitschrift für Physik 155, 1959

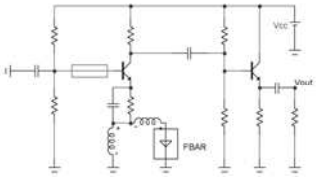
- Suitable materials (e.g. AlN) in principle compatible with CMOS processes and already in use for other MEMS applications.
- Operating frequencies in the low GHz range.

Gravimetric Sensors
FBAR Proof-of-Principle: BSA Detection



Measured shift of resonance frequency





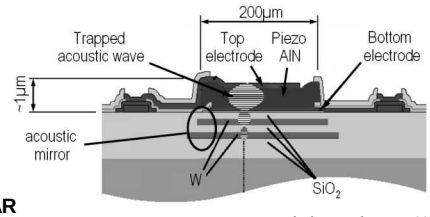
- **Sensor mounted on PCB and operated with oscillator circuit consisting of discrete components here.**
- **CMOS integration: cf. next slides.**

R. Breferlow et al., IEDM 2003

Gravimetric Sensors
CMOS on FBAR Chip

Basic FBAR Technology

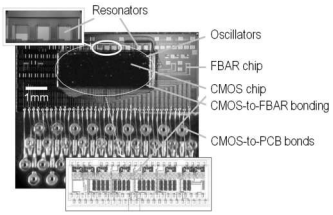
- Si substrate
- AlN used as piezoelectric
- acoustic mirror realized by buried W layers
- Δm deposited on top electrode

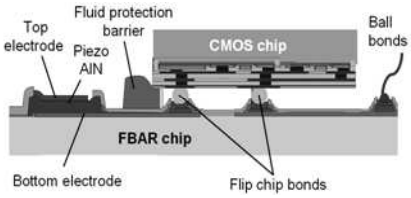


R. Brederlow et al., IEDM 2003

Flip-Chip Bonding CMOS-to-FBAR

Face-to-face flip chip bonding of 0.13 µm standard CMOS and FBAR chip
→ short interconnects between oscillator circuit and resonator



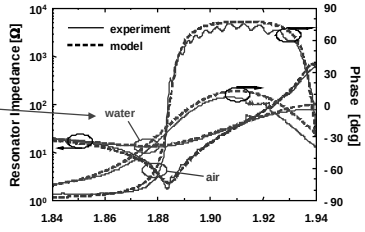


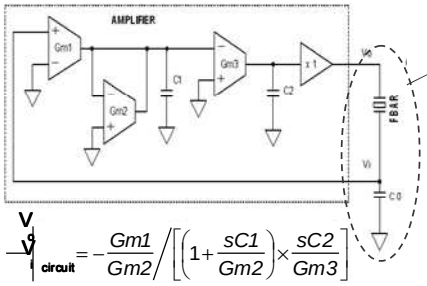
M. Augustyniak et al., ISSCC 2007

Gravimetric Sensors
FBAR - Oscillator Design

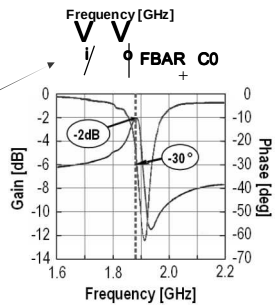
Challenge:
low Q-factor in water

Requirement:
precise gain / phase relationship (+5dB / 330°) at resonance frequency (1.86GHz)





$$\frac{V_o}{V_i}\Big|_{circuit} = -\frac{Gm1}{Gm2} \Big/ \left[\left(1+\frac{sC1}{Gm2}\right)\times\frac{sC2}{Gm3}\right]$$



M. Augustyniak et al., ISSCC 2007

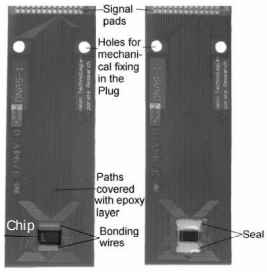
Outline (Part II)
CMOS DNA Microarrays: Circuit and System Aspects

Assembly and Packaging Aspects

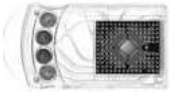
- Packaged electronic biochips require a fluidic and an electrical interface. Interfacing effort in case of optical biochips (fluidic + optical interface) is usually lower!
- Electronic biochips: Cheap and reliable packaging solution required.
- Requirements concerning in-package (micro-) fluidics:
  - 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.

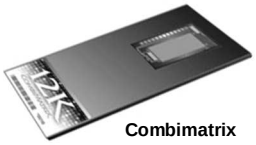
Chip Assembly
Examples: Published Assembly / Packaging Approaches



Infineon (until 2005)



Nanogen



Combimatrix

Siemens (under development)

- "open system"
- simple fluidic interface
- PCB-based electrical interface

- fluidic interface with in-package microfluidics
- electrical interface with in-card electrical interconnects

- fluidic + electric interface with in-package microfluidics and in-card el. interconnects
- in-card stored bio-chemical compounds

Complexity

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

4. Conclusion

CMOS-based bio-molecule microarrays have been discussed considering applications and basic operating principles, CMOS extra processing requirements, circuit and system design issues.

Standard CMOS processes require process extensions which must not deteriorate CMOS frontend properties. Various extended CMOS processes have been demonstrated to be feasible.

From the user's point of view the entire system (including assembly, packaging, storage, microfluidics, software, ..., target application and related assays) must be considered.

Commercialization is on the way in some cases as CMOS chips have proven their capability to open novel and user-friendly solutions. The full potential of CMOS-based biosensor arrays, however, is still under development as well as appropriate business models.

Thank you!