On-chip DNA electrical detection based on Si-CMOS compatible Al capacitors and inductors coated with metal oxides

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Objectives

DNA detection on silicon chips?

- Substitute fluorescent labeled DNA probe optical imaging, by electrical measurements: impedance spectroscopy, AC current or capacitance, DC current or resistance, photocurrent, etc.
- Lower cost, high throughput
- Si-CMOS mainstream technology for IC integration
- Fast point-of-care diagnosis applications
- Future lab-on-a-chip miniaturization
State-of-the art : Electrical detection

1.1. Unlabeled DNA, real time

(1) Direct impedance sensing
(e.g. Yi et al. 2004)
- Micromachined 1.5µm x 60 nm gaps
- Label-free 0.5 µM ssDNA targets = 30 % capa change in solution
1.1. Unlabeled DNA, real time

(2) Field-effect transducing
(e.g. Fritz et al. 2002)
- Cantilevers (500 µm-long) for insertion inside microchannels, supporting 50 x 50 µm² EIS (positively-charged polymer + thin SiO₂ + Si channel + contact)
- 2 nM ssDNA targets = 0.2 mV of threshold voltage shift (compared to no-match) in solution

1.2. Labeled DNA, real time

Electrochemistry
(e.g. Dill et al. 2004, using CombiMatrix Pt-coated chips)
- Enzyme labels catalyze redox reactions of solution reagents under low voltage (< 1 V in this case)
- 0.1 pM ssDNA targets ~ 0.5 nA DC current in solution
1.3. Labeled DNA, post-amplification (1)

**State-of-the-art: Electrical detection**

**Conductimetry**
- (e.g. Park et al. 2002; Li et al. 2003)
- Enhanced metal colloid aggregates between noble-metal electrodes
- Measurements *out of a solution*
- With ~1 pM ssDNA targets, hundreds of mega-Ohms initial resistance falls down to kilo-Ohms

**State-of-the-art: DNA binding**

**Functionalization (trichloro-silane)**

**DNA probes spotting (covalent bonding)**
State-of-the art: Silver amplification

1. Hybridization (selective bonding)

2. Gold labeling (20-100 nm balls)

3. Amplification 40x (Ag grains 1 µm)

4. Anti-biotin antibodies coupled to gold nanoparticles

5. Silver enhancement

State-of-the art: Electrical detection

1.3. Labeled DNA, post-amplification (2)

Limitations/problems:
- Noble metal required (non-CMOS standard or compatible, higher cost)
- Au catalyzes Ag precipitation
- Large gaps needed (20 µm)
- Long enhancement time (35 min.)
- Successive solution replacements (10-15 times)
- Hazards of false or non-selective detection?
- No reference for calibration, Reproducibility? (Multiple current paths with different R values can appear for the same reaction)
CMOS materials and DNA compatibility

2.1. Si-CMOS technology and materials

Cross-section of Si-CMOS wafer

- **Silicon** doped with various impurities
- **Metals** (Al, Cu, W)
- **Poly-Si**
  - Or **Ti/Co/Ni silicides**
- **Dielectrics**:
  - Si oxides
  - PECVD or PYROX
  - Wet
  - Dry
  + Si nitride
  + polymers

CMOS materials and DNA compatibility

2.2. DNA binding on Si oxides

Fluorescence imaging of aminated Cy3-DNA spotted on

- **Dry SiO$_2$**
  - functionalized with trimethoxysilane in toluene
- **Wet SiO$_2$**
- **PECVD**
- **Glass**

Better results with wet SiO$_2$ and aldehyde functionalization
CMOS materials and DNA compatibility

But:
- Top oxide = PECVD or PYROX, not wet SiO$_2$

• Trimethoxysilane functionalization less homogeneous than more usual trichlorosilane

2.3. Biocompatibility / resistance of top Si oxides

Al lines covered with densified PECVD oxide
But attacked by HCl release of trichlorosilane functionalization

Need for hard coating, i.e. chemically inert, but thin, i.e. transparent to electrical signals, & with DNA binding sites!
2.4. New concept

Metal oxide coating of Al electrodes

- Protection against chemicals/subproducts during the biological process
- OHs terminals in the surface to allow the functionalization
- Reduction of non-specific Ag precipitation
- System change from resistive to capacitive

2.5. Fabrication process

- Std Cleaning
- Wet Oxidation
- Al deposit & Lift-Off
- Bulk boron doped Silicon wafer
- SiO₂
- Photoresist
- Al
CMOS materials and DNA compatibility

2.5. Fabrication process

- 2nd Metal Deposition
- Anodization
- Back Side Prep

2nd Metal Deposition
Al or Ti
Metal Oxide
Aluminum

2.6. Anodization process

Anode: $2\text{Al}^{3+} + 3\text{OH}^- \rightarrow \text{Al}_2\text{O}_3 + 3\text{H}^+$ (CMOS wafer)

Cathode: $2\text{H}^+ + 2e^- \rightarrow \text{H}_2\uparrow$ (reference electrode)

Chemical solution to form oxide:

- **Non-porous**: Boric acid/etilenglycol
- **Porous**: Sulfuric acid or Phosphoric acid in water
CMOS materials and DNA compatibility

2.7. Anodization binding results

Spots After Silver Precipitation

Biotinylated DNA

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Glass</th>
<th>Wet SiO₂</th>
<th>Al₂O₃ (anodized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.78 nM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.56 nM</td>
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<td></td>
<td></td>
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<tr>
<td>3.12 nM</td>
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<td></td>
</tr>
<tr>
<td>6.25 nM</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12.5 nM</td>
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<td></td>
</tr>
<tr>
<td>25 nM</td>
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<td></td>
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<tr>
<td>50 nM</td>
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<td></td>
</tr>
<tr>
<td>100 nM</td>
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<td></td>
<td></td>
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<tr>
<td>200 nM</td>
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</tbody>
</table>

SiO₂, Al₂O₃ ≈ Glass

Capacitive detection of Ag-enhanced DNA

3.1. Interdigitated electrodes (IDE) : Basics

R, C depend on frequency And on Ag density (i.e. DNA)
3.2. Interdigitated electrodes (IDE):

2-D Simulation of electrical coupling

80% of current lines flow in a layer of thickness $L/2$, where $L$ is the sum of a finger width and adjacent spacing.

Optimal dimensions
Ag grain diameter 1 µm

3.3. Interdigitated electrodes (IDE):

Simulation of random Ag-grains distribution between electrodes

More sensitive for closer electrodes

Frequency 1kHz...10MHz
Capacitive detection of Ag-enhanced DNA

3.4. Interdigitated electrodes (IDE) : Experiments

- well-defined spot coverage
- low background noise
- DNA binding & Ag-grains as expected on top of and between electrodes

1° Functionalization: aldehyde formation
2° Spotting: 20nM solution of HIV biotinylated dsDNA
3° Ag precipitation: with hydroquinone (Silver Blue solution, Eppendorf Array Technologies, Namur, Belgium)

Optimization of Ag-enhancement duration

- between 2 and 7 min.: low background noise
- above 7 min.: short-circuits
3.4. Interdigitated electrodes (IDE) : Experiments

- spotting with biotinylated dsDNA ranging between 0 and 20 nM
- Ag-revelation : 2.5 min.

![Graph showing capacitance vs. frequency for different DNA concentrations.]

Silver crystals coverage of 10-20%, 30-40% and 60-80% from top to bottom

<table>
<thead>
<tr>
<th>DNA concentration</th>
<th>0.2 nM</th>
<th>2 nM</th>
<th>20 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrodes structures</td>
<td>1µm</td>
<td>2µm</td>
<td>3µm</td>
</tr>
<tr>
<td>10-20% coverage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40% coverage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-80% coverage</td>
<td></td>
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</tbody>
</table>
3.4. Interdigitated electrodes (IDE) : Experiments

- The higher concentration, the higher capacitance.
- Capacitance values higher with 1 µm finger size.
- Below 1 nM, 1 µm spacing is better for detection.
- Concentrations down to 0.2 nM could be detected.

Microwave detection

4.1. Single-level Meander Inductors : Definitions

RLC circuit Resonance frequency: “the one at which the inductor behavior changes from inductive to capacitive”, i.e. at the change of the sign of the complex part of the impedance seen at the input

\[ f_{res} \approx \frac{1}{2\pi} \cdot \frac{1}{\sqrt{L_s C_s}} \]

Decreases with Ag-ampli.
4.1. Single-level Meander Inductors : Results

Measured Y-parameters (reflection (top), transmission (bottom)) versus frequency before (solid red) and after hybridization (dashed blue) DNA 20nM.

- Frequency Shift on alumina < titanium oxide

\[ C_S \text{ after hybridization} = f(\text{thickness, permittivity}) \]

- TiO\(_2\) 20 nm \(\varepsilon \gg 15\)
- Al\(_2\)O\(_3\) 30 nm \(\varepsilon = 8\)

- From Resonance Frequency shift:

<table>
<thead>
<tr>
<th>Material</th>
<th>(C_S) variation</th>
<th>Percentage (before-after hybr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al(_2)O(_3)</td>
<td>15.2 fF</td>
<td>80 %</td>
</tr>
<tr>
<td>TiO(_2)</td>
<td>16.7 fF</td>
<td>95 %</td>
</tr>
</tbody>
</table>
4.2. Two-level Spiral Inductors : Results

Spotting : 375 nucleotide-long biotin-labeled ssDNA (Eurogentec, B)
Hybridization : 5 nM comp. ssDNA
Silver precipitation

Quality factor

before

after

Transmission Y parameter

before

after

On-chip detection circuit can be designed using mainstream comm IC blocks with resolution < 50MHz, i.e. < 0.2nM
UV detection with thin-film SOI Pin diodes (1)

UV photodiode

with QE > 60 %
For $\lambda < 400$ nm
And $t_{Si} = 80$nm (w/o ARC)
+ very low dark current

Quantum efficiency (%)

Co-integrated read-out
Similar to CMOS camera

SOI-CMOS 2µm: 10kHz sampling,
1:7000 dynamic range,
Power=5nW/pixel

UV detection with thin-film SOI Pin diodes (2)

Ag-enhanced DNA spots on glass slides over our photodiodes

Ag-enhanced DNA spots on glass slides over our photodiodes

Init.
0.4 nM
4 nM
20 nM

$\lambda = 370$nm
Conclusions (1)

Compatibility and advantages of metal oxide coating

- Protection: standard low-cost silicon IC fabrication processes with non-noble metals can be used;
- Reduction of parasitic silver precipitation on the electrodes: use of short gaps between them and shorter silver reaction times with lower probability of false detection;
- Less spaced gaps: potential for higher sensitivity, lower levels and better quantification, and repeatability of the detection;
- Capacitive/inductive measurement: calibration of the sensor at a reference level before or in the absence of hybridization, on the contrary to resistive values which tend to infinity and are hence limited by the measurement set-up.

Conclusions (2)

DNA detection is possible through the use of passive integrated resonant circuits and IDEs capacitors over a wide frequency band (from 10 KHz to 40 GHz). We also demonstrated that sensitivity could be increased using thinner spaces between lines or fingers and using CMOS compatible metal oxides with high dielectric constant (e.g. Al₂O₃ and TiO₂).

Since detection of a shift of 50 MHz for the self-resonance frequency is possible using basic microwave electronics, which would correspond to a $C_s$ change of less than 0.1 fF, it is expected that very low concentrations (less than 0.2 nM) will be detectable.