Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications

Sven Ingebrandt

University of Applied Sciences Kaiserslautern – Campus Zweibrücken, Germany
Outline

- Introduction to the University of Applied Sciences Kaiserslautern
- Motivation
- Silicon nanowire (SiNW) detection principles
- Top-down fabrication of SiNW sensors
- Electronic readout principles for SiNW sensors
- Electrochemical characterization
- Bioassays with SiNW sensors:
  1. DNA immobilization and hybridization detection
  2. Ca\(^{2+}\) ions – peptide interaction
  3. Immunoassays
  4. Detection of cellular signals
  5. Impedimetric detection of cellular adhesion
- Possible future assays with SiNW sensors
- Summary
Introduction to the University of Applied Sciences Kaiserslautern
Introduction to the UASK: Zweibrücken

Area: 70.64 km$^2$
Population: 34,109

City of roses and horses
Introduction to the UASK

- Spread over three cities Kaiserslautern, Pirmasens, and Zweibrücken
- 5 faculties, 23 courses, 6000 students, ~168 professors

Campus Kaiserslautern
- Applied engineering sciences
- Construction and design

Campus Pirmasens
- Applied logistics- and polymer-sciences

Campus Zweibrücken
- Economics
- Informatics and Microsystemtechnology
Introduction to the UASK:
Courses with subjects Micro-Nano-Bio in our faculty I/MST

„Applied Life Sciences: Applied Bio-, Pharma- and Medical Sciences“

Bachelor of Science (7 Semesters)

Master of Science (3 Semesters)

„Microsystem- and Nanotechnology“

Bachelor of Engineering (7 Semesters)

Master of Engineering (3 Semesters)
Introduction to the UASK: Study in Zweibrücken

Speciality: Large clean room (300 sqm) for education and research

Student courses / Summer schools
Introduction to the UASK: New highlight - Nanoimprint Lithography

DFG-'Large equipment proposal: 
Obducat Eitre 6“
Nanoimprint-Lithography

Installed in cleanroom of FHKL
Introduction to the UASK: New highlight - Schottky Field Emission SEM

Zeiss Gemini Supra with high Resolution EDX
Introduction to the UASK: Research in the department I/MST

Research Focus IMS (integrated miniaturized systems)

http://www.fh-kl.de/fh/forschung/forschungsschwerpunkte/ims.html

AG Ingebrandt
AG Moebius
AG Müller
AG Saumer

Involved Professors
Introduction to the UASK: Ph.D. students in the department I/MST

At the moment 22 cooperative Ph.D. theses:

8 in AG Ingebrandt
4 in AG Schäfer
3 in AG Moebius
1 in AG Hoffmann

4 in AG Müller
1 in AG Saumer
1 in AG Picard
International Connections

Cooperations:

- Chinese University of Hong Kong (China)
- Universität Hasselt (Belgium)
- University of Florida (USA)
- Université catholique de Louvain (Belgium)
- Ha Noi University of Technology (Vietnam)
- Hanyang University Seoul (South-Korea)
- Cranfield University (UK)
- National Research Council, Ottawa (Canada)
- KAIST, Daejeon (South-Korea)
- RIKEN, Tokyo (Japan)
- University of Sydney (Australia)
- .......

30% of all students go abroad, e.g. practical phase or bachelor thesis in:

- Edinburg, UK
- Hyderabad, India
- Gainesville, Florida
- Tokyo; Japan
- Singapore
- Ha Noi, Vietnam
- ...

Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications - Sven Ingebrandt
Introduction to the UASK: Research in the department I/MST

Conferences organized by our university:

AG Ingebrandt 2012 in Cancun, Mexico

EnFI 2012 Conference in Zweibrücken
AG Biomedical Signalling - Group structure
Prof. Dr. Sven Ingebrandt
Walid-Madhat Munief (Assistant)
Nina Stock (Technical assistant)

<table>
<thead>
<tr>
<th>Biosensor Principles</th>
<th>Cell-Sensor Hybrids</th>
<th>Micro- and Nanodevices</th>
<th>Nanoscale Transducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group head:</td>
<td>Group head:</td>
<td>Group head:</td>
<td>Group head:</td>
</tr>
<tr>
<td>Dr. Maryam Weil</td>
<td>Dr. Jessica Ka-Yan Law</td>
<td>Dr. Xuan-Thang Vu</td>
<td>Dr. Vivek Pachauri</td>
</tr>
<tr>
<td>Ph.D. students:</td>
<td>Ph.D. students:</td>
<td>Ph.D. students:</td>
<td>Ph.D. students:</td>
</tr>
<tr>
<td>Lotta Delle</td>
<td>Anna Susloparova</td>
<td>Miriam Freyler</td>
<td>Ruben Lanche</td>
</tr>
<tr>
<td>Students:</td>
<td>Dieter Koppenhöfer</td>
<td>Thanh Chien Nguyen</td>
<td>Xiaoling Lu</td>
</tr>
<tr>
<td>Tina Welsch</td>
<td>Students:</td>
<td>Dipti Rani</td>
<td>Students:</td>
</tr>
<tr>
<td>Alice Kasjanow</td>
<td>Nathalie Schüssler</td>
<td>Darja Schendel</td>
<td>Jan-Felix Schoppmeier</td>
</tr>
<tr>
<td></td>
<td>Olga Fominov</td>
<td>Stefan Ohlinger</td>
<td>Claudia Ackermann</td>
</tr>
</tbody>
</table>
Biomedical Signalling Research Group (AG Ingebrandt) – July 2013
Motivation
Motivation: Biosensors and applications

Applications
• Food analysis
• Drug development
• Criminal investigation
• Medical diagnosis
• Test of donated organs

Ideal properties:
• High sensitivity
• High selectivity
• Cost effective devices
• Real-time detection
• Fast response time
• Point-of-care usage, …

Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications - Sven Ingebrandt
Motivation: ISFET-based biosensors – General principle

- Label – free and real-time detection
- Direct electronic readout signal
- Possibility of a miniaturization

\[ V_{TH} = E_{ref} - \Psi_S + \chi_{sol} \frac{\Psi_{Si}}{q} - \frac{Q_{ox} - Q_{ss}}{C_{ox}} - \frac{Q_B}{C_{ox}} + 2\Phi_F \]
Detection principles: Potentiometric measurements

FET as potentiometric biosensor

• The gate potential is **modulating the inversion channel** between drain and source
• **Changes in drain-source current** are proportional to changes of the **gate potential** (in the linear region)

**Two signal components:**

a) **Potential change** at the gate caused by an **action potential of a cell**

b) **Potential change** at the gate caused by changes of the ionic concentration of the electrolyte near the gate. e.g. **pH-sensitivity**
Detection principles: Impedimetric measurements

ISFET as impedimetric biosensor – Transistor-Transfer Function (TTF)

Transfer Function:

\[ H(\omega) = \frac{V_{\text{out}}(\omega)}{V_{\text{stim}}(\omega)} \]

Low cutoff frequency

\[ \tau_1 = R_{\text{mem}}(C_{\text{mem}} + C_{\text{ox}}) \approx R_{\text{mem}}C_{\text{ox}} \]

High cutoff frequency

\[ \tau_2 = R_{\text{mem}}C_{\text{mem}} \]

Schasfoort RBM et al., (1989) Sensor Actuator, 18, 119
Bio-sensing applications: Electronic DNA-chip with micro-ISFETs

S. Ingebrandt, Y. Han, F. Nakamura, A. Poghossian, M.J. Schöning, and A. Offenhäusser. 
*Label-free detection of single nucleotide polymorphisms utilizing the differential transfer function of field-effect transistors*, 
*Biosensors and Bioelectronics*, In Press, Corrected Proof, Available online 21 December 2006

- Novel, lock-in based signal transduction concept
- Reliable recording
- Fast sampling
Motivation: Silicon nanowire biosensor

Advantages

- Ultrahigh surface-to-volume ratio
- Dimensions of the biomolecules are comparable to that of the nanowires
- Strong influence of the surface effects to the electronic properties
  
  High sensitivity

- Possibility to create dense arrays

Requirements

- Wires in the 10 nm range with good carrier mobilities
- Low density of trap states at the interfaces (high subthreshold swing)
- High transconductance
- Stability and reliability of the output signal
- Processability of the sensors
Top-down fabrication of SiNW arrays
Fabrication process of Si-NW arrays

- First trial: Electron beam lithography

At project start: Si nanowire by e-beam lithography and RIE (in cleanroom of IBN-2)

- Electron Beam Writer Leica EBPG 5000 Plus

- High resolution (down to 5nm)

- Maskless patterning

Bad surface quality
Strong hysteresis
Slow process
Fabrication process of Si-NW arrays

- Silicon nanowire device concept:
  - Reed group (Yale)
    - Top-down approach
    - Biomolecular sensing demonstrated recently

[Source: Stern er al, Nature, February 2007]

Wet etching process with tetra methyl ammonium hydroxide (TMAH)
Wafer-scale fabrication of SiNW arrays

1. Start: SOI substrate
2. Thin out of top Si
3. Nanoimprint lithography
4. RIE etching of oxide mask
5. TMAH etching of Si
6. Contact line implantation
7. ONO passivation
8. RIE etching and metal lift-off

Si, p-Si, SiO2, Si3N4, Resist, Metal

After TMAH etching
After remove SiO2

Chip designs

4×4 Arrays
6 wires parallel
Length: 3mm
Width: 100, 200, 500, 1000nm (mask design)
pitch: 200mm

28×2 Arrays
Single wires
Length: 10mm, 20mm, 40mm
Width: 200, 400nm (mask design)
pitch: 50mm or 10mm

Integration with micro-fluidics

16×16, 32×32 and 128×128 arrays


Fabrication process of Si-NW arrays

Fabrication of larger Si-NW FET arrays

Two designs:
16×16 with 100 μm pitch (256 channels)
32×32 with 50 μm pitch (1024 channels)

• PECVD passivation was done in Zweibrücken
Fabrication process of Si-NW arrays: SiNW devices

- Wafer-scale, reproducible process
- Size of SiNW can be as small as 60 nm at the bottom (in current process)
- High quality of the gate oxide and smooth surfaces
- High quality of the passivation layer – stable operation in electrolyte solution
Fabrication process of Si-NW arrays: Wafer-scale processing

4 inch wafer

28x2 arrays

128x128 arrays
NEW Fabrication process of Si-NW arrays in Zweibrücken has started

Mold: nanowire structure

Imprint: nanowire structure in PMMA
NEW Fabrication process of Si-NW arrays in Zweibrücken has started

The photographs show:

- SEM image of nanowire structure etched into SiO₂
- AFM image of PMMA imprinted nanowire structure
Fabrication process of Si-NW arrays: Chip packaging

- Wire – bonding
- Contact isolated: Medical epoxy, PDMS
- Can be used directly or integrated with micro-fluidics
Portable amplifier for the FET arrays

- Simultaneous measurement of all 16 channels
- Precise temperature control
- Amplifier stages with 1x, 10x, 30x, 100x
- Differential readout with reference channel
- Measurement of the transfer function by a 16x lock-in circuit
- Portable system with USB-interface to PC
- Custom-made software (Delphi 5.0)
- Sampling rate 1 Hz (up to 10 kHz per channel with external USB-DAC card)
NEW 32-channel readout system design

- Here is the design for one channel, different channels are currently addressed by switching
- The system is flexible for different ranges of drain-source currents
- Suitable for both characterization and impedimetric measurement of FET devices

NEW amplifier system

LabVIEW programming

- State machine programming:
  - Program is divided into different states
  - Easier to read
  - Easier to manage
  - Easier to scale
- Stand-alone application: no LabVIEW development tool is needed

Headstage setup

At the moment still single channel

NEW labview-based readout software
Electrochemical characterization
Electrical characterization setup

- Understanding the SiNW electrical transport properties
- Finding of a stable configuration for biosensor experiments

Back - gate characteristics: $I_{DS}(V_{BG})$

Front gate in air:
- Front gate with electrolyte (floating potential or applied voltage)

Front - gate characteristics: $I_{DS}(V_{FG})$

Back gate floating
- Back gate with applied voltage
### Electrical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Implanted CL</th>
<th>Non-implanted CL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Back-gate control</strong></td>
<td>p-type only unstable operation</td>
<td>p- and n-type unstable operation</td>
</tr>
<tr>
<td>(in dry and wet environment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Front-gate control</strong></td>
<td>p-type only stable operation</td>
<td>n-type only stable operation</td>
</tr>
<tr>
<td>(in wet environment)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Front-gate configuration was identified for optimum biosensing experiments
- In any case, an electrochemical reference electrode is needed for the front gate contact to keep the system stable and the readout signal reliable
Electrical characteristics

Front-gate transfer characteristics: $I_{DS}(V_{GS})$

- **P-** enhancement and long-channel transistor
- $V_{TH} = -0.8\ V$ at pH 7
- $g_{m,\max}$ (at $V_{DS}=-2V$): 0.5 $\mu$S to 10 $\mu$S
- $I_{on}/I_{off} = 10^5-10^6$
- $S = 80 - 110\ mV/\text{decade}$, size dependent
Electrochemical characterization

pH sensitivity: Two possible operation modes

Saturation region

Sub-threshold region


pH sensitivity: 42 mV / pH and does not depend on size and operation mode
Bioassays

Assay 1: DNA detection
Assay 1: DNA immobilization and hybridization

SiNW
Immobilization: $\Delta V_G = 1-10$ mV
Hybridization: $\Delta V_G = 1-4$ mV
Han et al. 2006

ISFET

SiNW sensor is much more sensitive than our microscale ISFETs
Assay 1: Covalent attachment of DNA probes

Protocol (a)*:
- Silanization with 3-aminopropyltriethoxysilane
- Usage of succinic anhydride as cross linker
- Covalent attachment of amino-functionalized DNA

Protocol (b)**:
- Silanization with 3-glycidoxypropyltrimethoxysilane
- Direct, covalent attachment of amino-functionalized DNA and of proteins

* Han et al., 2006; Ingebrandt and Offenhäusser, 2006.
**Ghosh-Moulick et al., 2009.
Assay 1: Surface modification - silanization

Gas phase silanization
- Homogenous monolayers
- Encapsulated material not damaged

Contact angle measurement
- Quantify wettability
- Qualify silanization:
  - Did silanization work?
  - Is layer homogeneous?
Assay 1: DNA microspotting & electrical readout

- Site-specific spotting of different capture molecules
- Differential read-out possible
- Defined distances to avoid cross-contaminations

Reference electrode holder

Source
Nanowire
Drain
Assay 1: Surface modification steps - overview

3-Glycidoxypropyltrimethoxysilane (GPTES)

- No cross-linker due to covalent binding
- Creation of monolayers

\[ \text{SiO}_2 \xrightarrow{\text{H}_2\text{SO}_4} \text{SiO}_2 \xrightarrow{\text{OH OH OH OH GPTES}} \text{SiO}_2 \]

\( \Rightarrow \) capture DNA 1
\( \Rightarrow \) blocking DNA
\( \Rightarrow \) target DNA 1

Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications - Sven Ingebrandt
Assay 1: DNA detection – dc readout

Applied concentrations:

- 1 µM capture DNA
- 0.5 µM cDNA
- 1 µM cDNA

V_TH shifted to the right due to binding of negatively charged DNA molecules

Results unpublished
Bioassays

Assay 2: Ca$^{2+}$ ions – peptide interaction
Assay 2: Ca\textsuperscript{2+} ions – peptide interaction

M. Hitzbleck et al., Submitted to Langmuir

Bioassays
Assay 3: Immunoassays
Assay 3: Antibody-antigen detection by SiNW (Human CNTF*)

- CNTF is known to alter the self – renewal process of neuronal stem cells and the progenitor cell division and differentiation
- It is used in the therapy and gene-therapy of retinal ganglion cell damage or loss
- CNTF seems to have a protective role in multiple sclerosis mouse model regarding demyelinisation and plays a role in the pathogenesis of other neurodegenerative diseases
- CNTF is a good model system for the future measurements of other neurotropic factors such as BDNF, GDNF, FGF and NGF
- CNFT is usually detected by an elaborated ELISA method
- Sensitivity of the ELISA method is 30pg/mL at a dynamic range of 30 pg/mL - 3000 pg/mL

*CNTF: Ciliary neurotrophic factor
Assay 3: Antibody-antigen – dc readout

- 1 µg/ml capture antibody
- 5 pg/ml BDNF
- Block with 1 % BSA
- 10 pg/ml BDNF
- 1 pg/ml BDNF
- 50 pg/ml BDNF

V_{TH} shifted to the left

Results unpublished
Bioassays

Assay 4: Detection of cellular signals
Assay 4: Recording of extracellular action potentials

Primary cardiac myocytes (E18) and HL-1 cells were cultured on NW-FETs arrays.

Once a confluent layer is formed cells are spontaneously beating.

Signals can be site-selectively recorded by the NW-FET array.

Rat cardiac myocytes at 5 DIV
Assay 4: Recording of extracellular action potentials

16-channel recording of action potentials

Signals show a similar shape as compared to ISFETs

Signal-to-noise ratio is improved compared to ISFETs

Assay 4: Recording of extracellular action potentials

Primary cardiac myocyte culture!!!

Each of the 16-channels is responding

High signal-to-noise ratio: 5 – 10 due to the strong coupling between the cells and the SiNWs
Bioassays

Assay 5: Impedimetric detection of cellular adhesion
Assay 5: Impedimetric detection of cellular adhesion

$V_{\text{stim}}$

1Hz – 1MHz

$V_D$

$I_{DS}$

$G_{FM}$

$C_{FM}$

$G_{NM}$

$C_{NM}$

$R_J$

$NW$

$Substrate$

$V_{DD}$

$V_{SS}$

$R_{FB}$

$C_{FB}$

$E_{PH}$

$C_{Gouy}$

$C_{Helm}$

$C_{drain}$

$G^*$

$R_{source}$

$R_{drain}$

$R_{out}$

$V_{G2} = 10\text{mV}_{\text{ac}}$

$\text{SiNW model}$

$\text{OPA627}$

$V_{out}$
Assay 5: Impedimetric detection of cellular adhesion

Results unpublished

HEK cell-SiNW coupling: Detailed SEM analysis
Future assays
New research projects related to SiNW sensing

- **Marie-Curie ITN: PROSENSE**
- **Cancer Diagnosis: Parallel Sensing of Prostate Cancer Biomarkers**
- **Start 01.10.2012**

<table>
<thead>
<tr>
<th>Participants</th>
<th>2 Ph.D. students (3 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Coordinator)</td>
<td>UBath</td>
</tr>
<tr>
<td>2</td>
<td>CardiffU</td>
</tr>
<tr>
<td>3</td>
<td>SAS</td>
</tr>
<tr>
<td>4</td>
<td>INESC-MN</td>
</tr>
<tr>
<td>5</td>
<td>DCU</td>
</tr>
<tr>
<td>6</td>
<td>AET</td>
</tr>
<tr>
<td>7</td>
<td>EPFL</td>
</tr>
<tr>
<td>8</td>
<td>FH-KL</td>
</tr>
<tr>
<td>9</td>
<td>Xeptagen</td>
</tr>
</tbody>
</table>

**Associated Partners**

| 1 | Euroimmun AG | Euroimmun | Germany |
| 2 | University Hospital of Wales | UHW | United Kingdom |
| 3 | University Hospitals Bristol National Health Service Foundation Trust | UHB | United Kingdom |
| 4 | University of the West of England | UWE | United Kingdom |
| 5 | North Bristol National Health Service Trust | BUI/NBT | United Kingdom |
| 6 | Royal United Hospital Bath National Health Service Trust | RUH | United Kingdom |

Subject 1: SiNW Sensors
Subject 2: rGO-Sensors
New research projects related to SiNW sensing

Developing a NW-based sensor platform for detection of PCa in parallel with fluorescence techniques
New research projects related to SiNW sensing

Si NW FET Fabrication on Sapphire substrate for combined optical and electrical sensing

- Dip Chip:
  - 32 single NW devices on 7*7mm² chip.
  - NW dimensions: 1µm(length)*0.1µm(width).
  - Common source configuration.
Summary

- Si nanowire arrays were successfully fabricated in a wafer-scale process. They can be highly integrated (128x128 arrays) and are CMOS compatible.
- Robust chips with reliable operation in liquid.
- DNA and biomolecular detection experiments were successful.
- Extracellular recordings are possible.

Outlook

- Sensitivity (DNA hybridization, protein, immune)
- Model to explain the results.
- Enzyme detection.
- Coupling with cells (mast cells, cardiac myocytes, tumor cell lines, neuronal cells).
Acknowledgements

FH Kaiserslautern
All members of AGBM and technical staff of FHKL

Cooperation partners

FH Kaiserslautern
Profs. Karl-Herbert Schäfer, Monika Saumer, Cornelia Keck

Research Center Jülich
Prof Dr. A. Offenhäusser

JL University Giessen
Prof. Dr. Martin Eickhoff

Phillipps-University Marburg
Prof. Dr. Wolfgang Parak

TU Kaiserslautern
Prof. Dr. Christiane Ziegler
Prof. Dr. Ing. Andreas König

University des Saarlands - Homburg
Prof. Dr. Markus Hoth

University of Applied Sciences Aachen
Prof. Dr. Ing. Michael J. Schöning

Universiteit Hasselt, Belgium
Prof. Dr. Patrick Wagner

The Chinese University Hong Kong
Prof. Dr. John A. Rudd
Prof. Dr. Chi-Kong Yeung

Nanyang Technological University Singapore
Prof. Dr. Peng Chen

Italian Institute of Technology - Genua
Dr. Axel Blau

Korean Advanced Institute of Science and Technology - Deajeon
Prof. Dr. Yoonkey Nam

Financial support:

BMBF:
‘Nanowire Sensors’
‘Cancer Cell Chip’
‘Multiparametric Sensing’

DAAD:
PPP exchange grant – Germany/Hong Kong

EU-ITN Marie Curie: Prosense

Industry: ‘Alternative Biosensor Principles’

Internal fh-funding:
- Nanotox
- Nanoimprint-Lithography

Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications - Sven Ingebrandt
Welcome to the website of the biomedical signalling group

Our interdisciplinary research group was founded in 2006 in the department of Informatics and Microsystems (IMST) at the University of Applied Sciences Kaiserslautern (campus Zweibrücken) and is headed by Prof. Dr. Sven Ingeberndt.

The research activities of our group are focused on the development of novel tools, technologies and methods for biomedical and pharmaceutical applications. We deal with development and fabrication of novel micro- and nanoscale chip based sensors. For instance, microelectrode arrays, silicon-based field-effect transistor arrays with different sizes from microscaled planar-MOSFETs to silicon-nanowire transistors. In parallel, we develop our own electronic amplifier and evaluation software for each type of devices. We apply these transducers for label-free detection of biomolecular interactions (e.g. DNA, antibody-antigen) and study of living single cells.