Top-Down Processed Silicon Nanowire Arrays for Biomedical Applications

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University of Applied Sciences Kaiserslautern –
Campus Zweibrücken, Germany

TU Dresden, 17.10.2013

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

Rosenstadt | ZWEIBRÜCKEN

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:

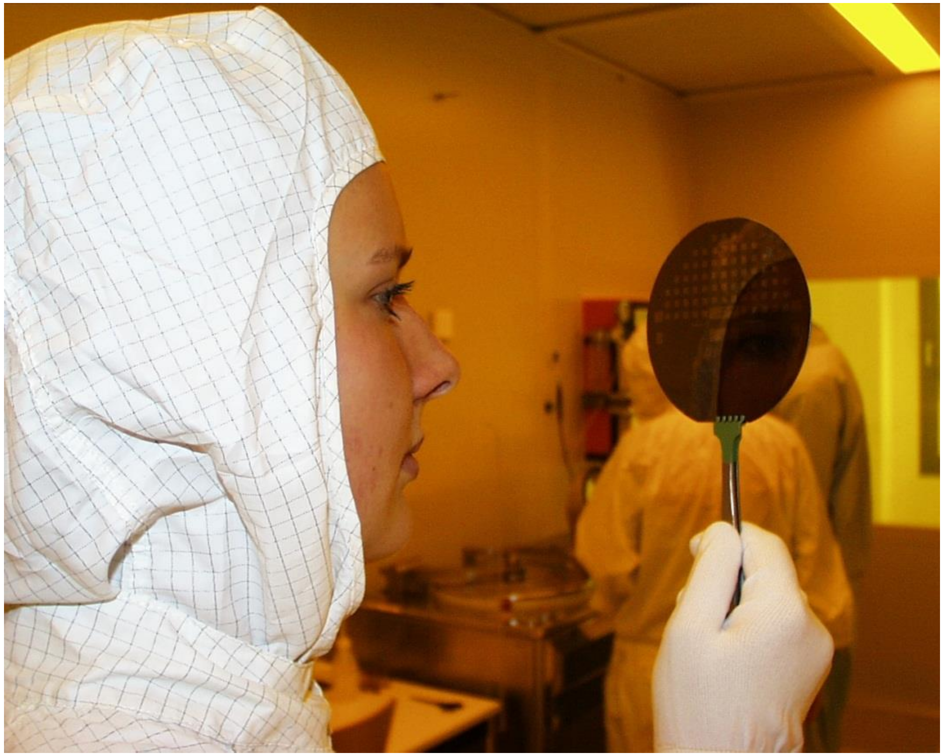
Courses with subjects Micro-Nano-Bio in our faculty I/MST

„Applied Life Sciences: Applied Bio-, Pharma- and Medical Sciences“	„Microsystem- and Nanotechnology“
Bachelor of Science (7 Semesters)	Bachelor of Engineering (7 Semesters)
⇓	⇓
Master of Science (3 Semesters)	Master of Engineering (3 Semesters)

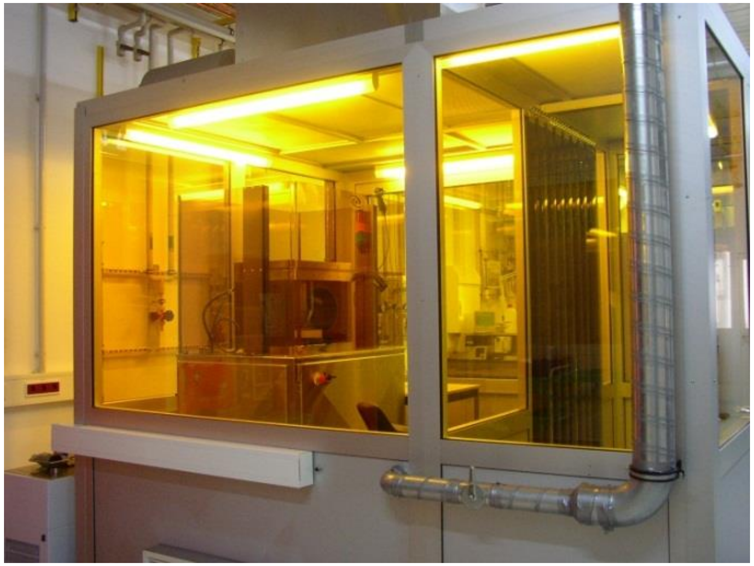
Introduction to the UASK: Study in Zweibrücken

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

Student courses / Summer schools

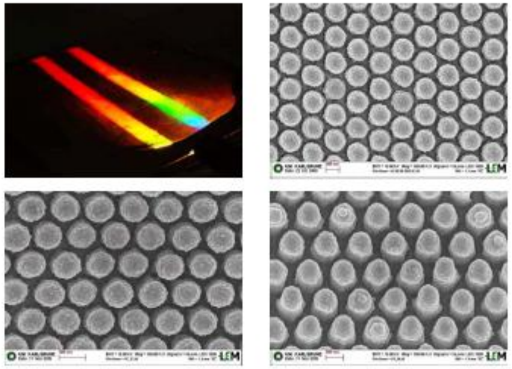


Introduction to the UASK: New highlight - Nanoimprint Lithography

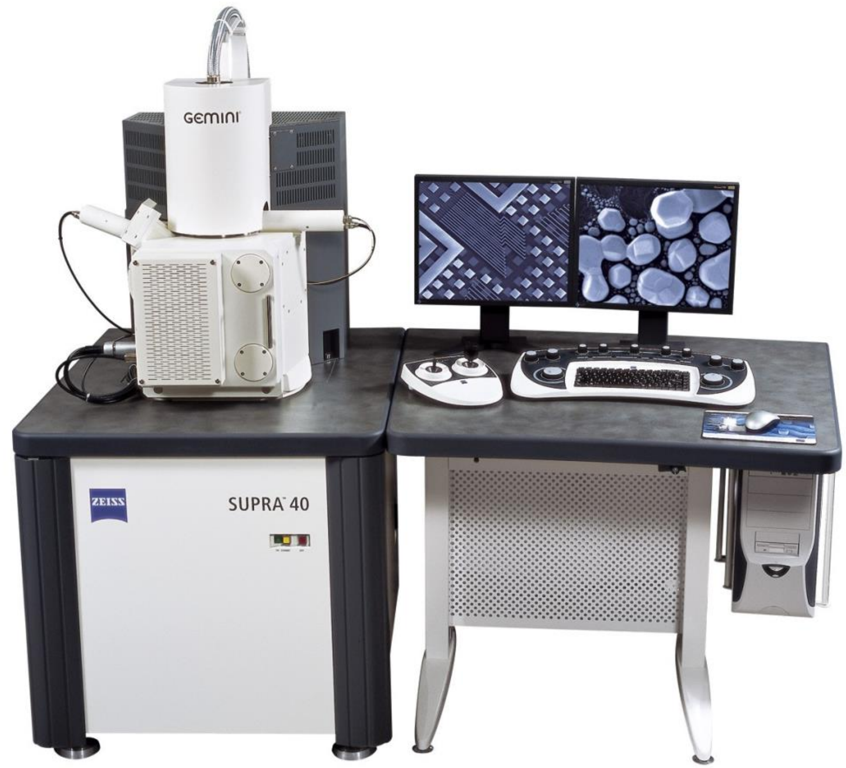


Installed in cleanroom of FHKL

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



Introduction to the UASK: New highlight - Schottky Field Emission SEM



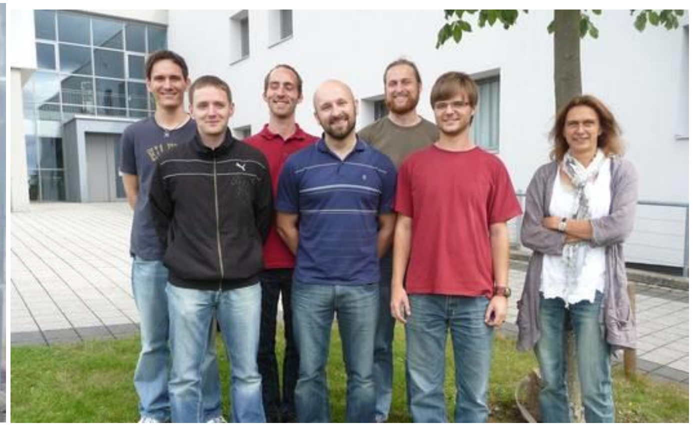
Zeiss Gemini Supra with high Resolution EDX

Introduction to the UASK: Research in the department I/MST

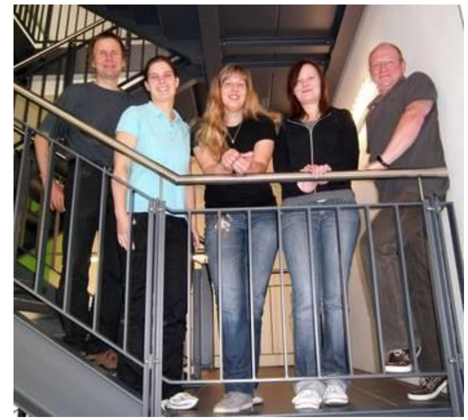
Research Focus IMS (integrated miniaturized systems)

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

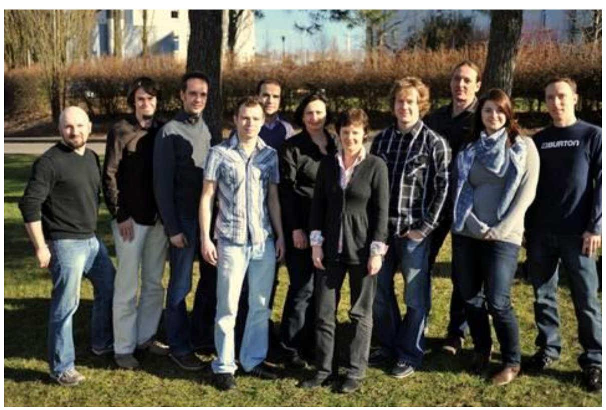
AG Ingebrandt



AG Moebius



AG Müller



AG Saumer

Involved Professors

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

Ph.D. students of the FB 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

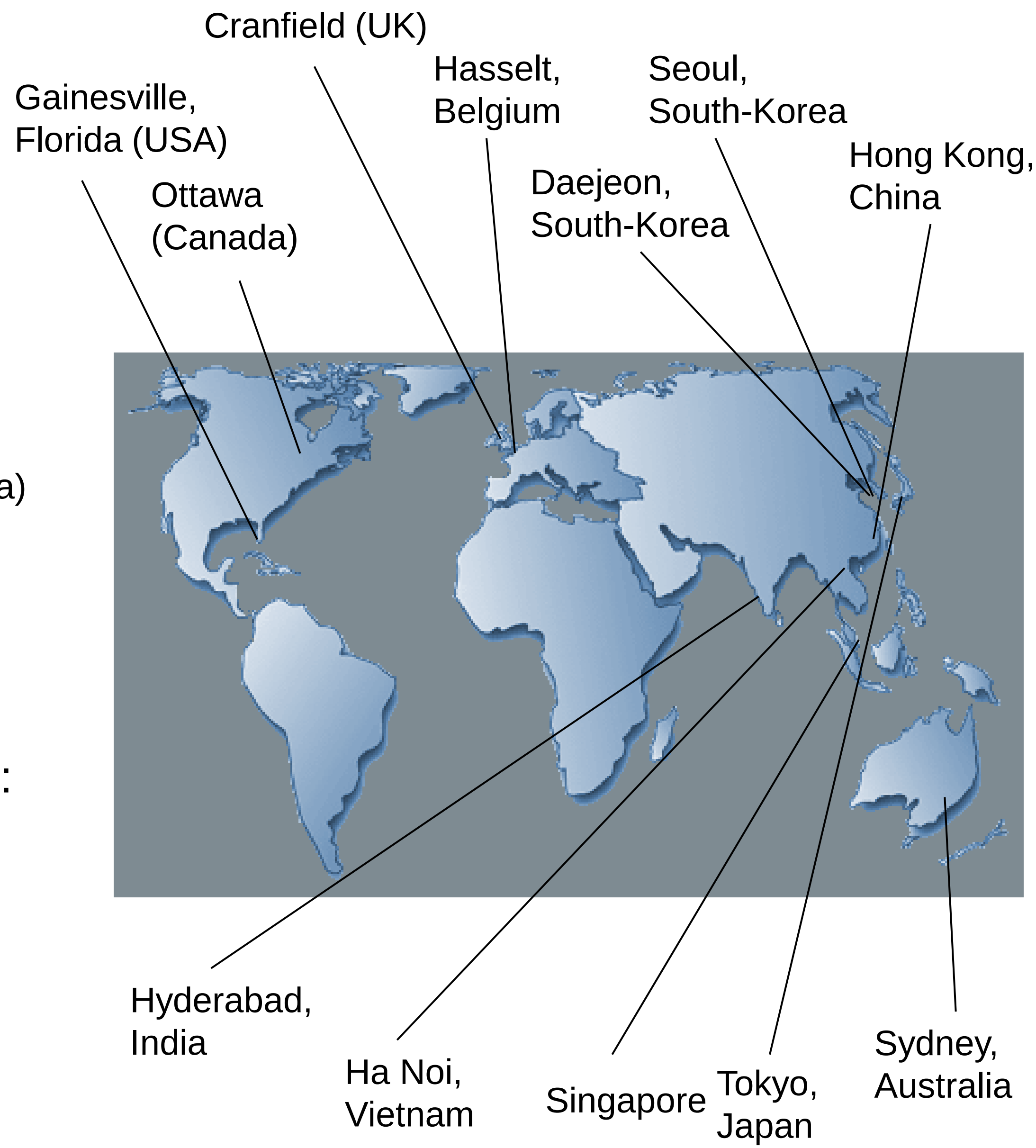
International Connections

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, Deajeon (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
- ...



Introduction to the UASK: Research in the department I/MST

Conferences organized by our university:

AG Ingebrandt 2012 in Cancun, Mexiko

EnFI 2012 Conference in Zweibrücken

AG Biomedical Signalling - Group structure

Prof. Dr. Sven Ingebrandt

Walid-Madhat Munief (Assistant)
Nina Stock (Technical assistant)

Biosensor Principles

Group head:

Dr. Maryam Weil

Ph.D. students:

Lotta Delle

Students:

Tina Welsch

Alice Kasjanow

Cell-Sensor Hybrids

Group head:

Dr. Jessica Ka-Yan Law

Ph.D. students:

Anna Susloparova

Dieter Koppenhöfer

Students:

Nathalie Schüssler

Olga Fominov

Micro- and Nanodevices

Group head:

Dr. Xuan-Thang Vu

Ph.D. students:

Miriam Freyler

Thanh Chien Nguyen

Dipti Rani

Students:

Darja Schendel

Stefan Ohlinger

Nanoscale Transducers

Group head:

Dr. Vivek Pachauri

Ph.D. students:

Ruben Lanche

Xiaoling Lu

Students:

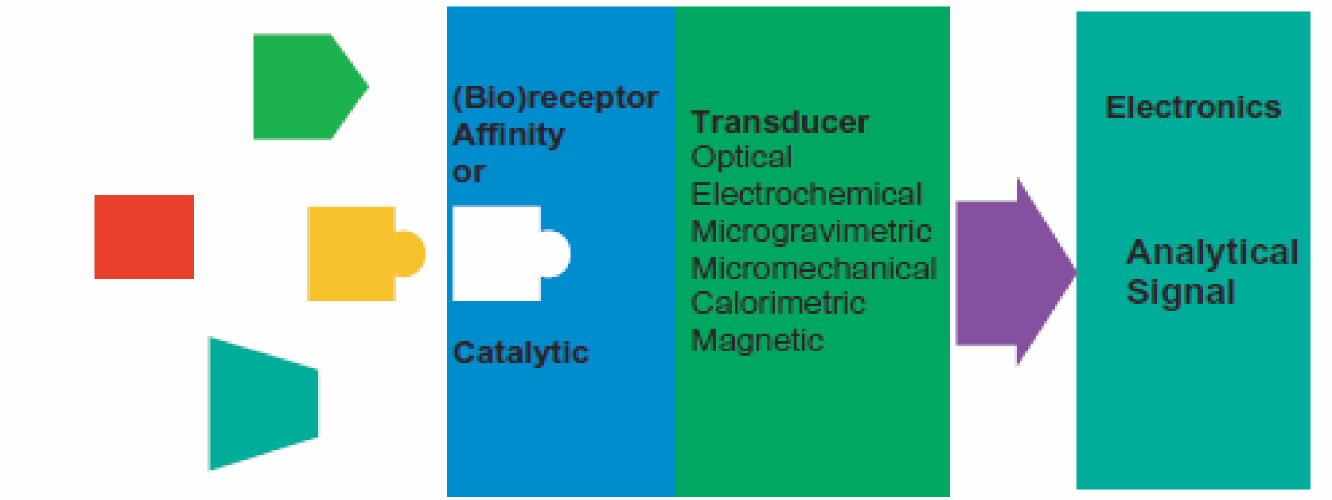
Jan-Felix Schoppmeier

Claudia Ackermann

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

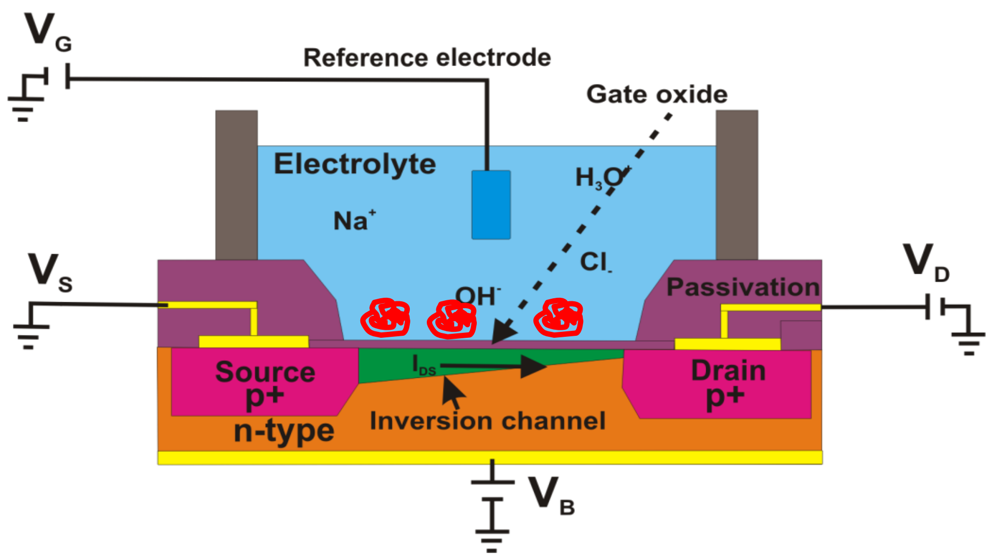
http://www.minimed.com

Ideal properties:

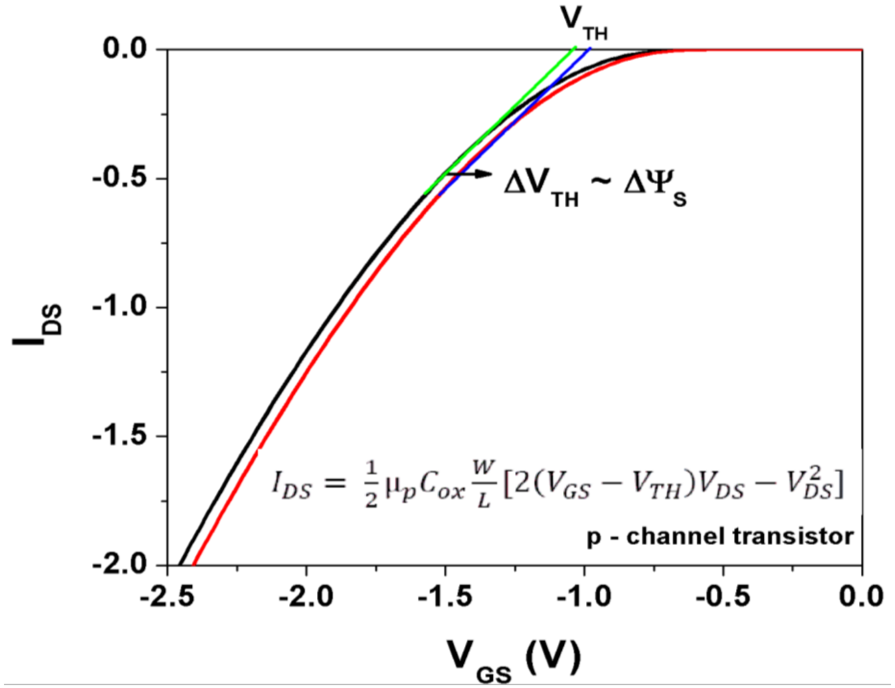
- High sensitivity
- High selectivity
- Cost effective devices
- Real-time detection
- Fast response time
- Point-of-care usage, …

Motivation: ISFET-based biosensors – General principle

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



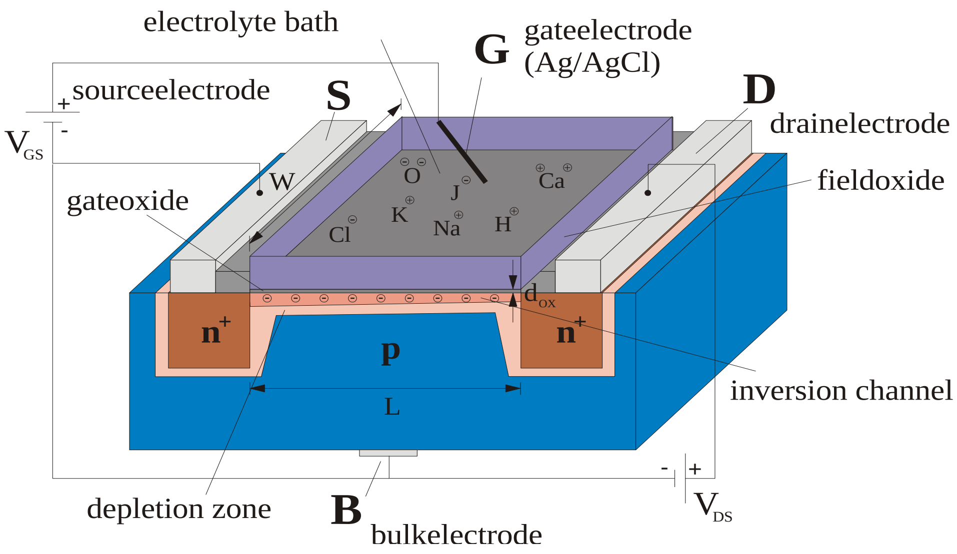
Transfer characteristics

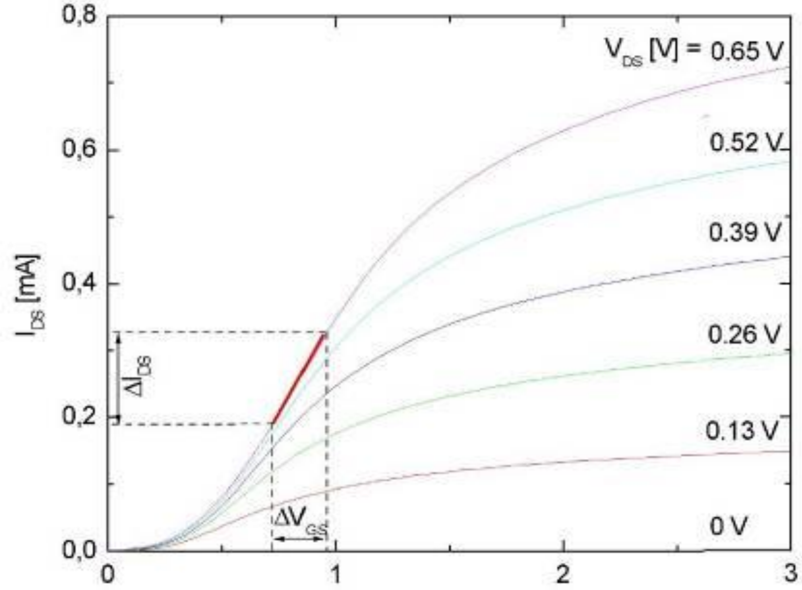


$$V_{TH} = E_{ref} - \Psi_s + \chi_{sol} - \frac{\Psi_{Si}}{q} - \frac{Q_{ox} - Q_{ss}}{C_{ox}} - \frac{Q_B}{C_{ox}} + 2\Phi_F$$

Detection principles: Potentiometric measurements

FET as potentiometric biosensor





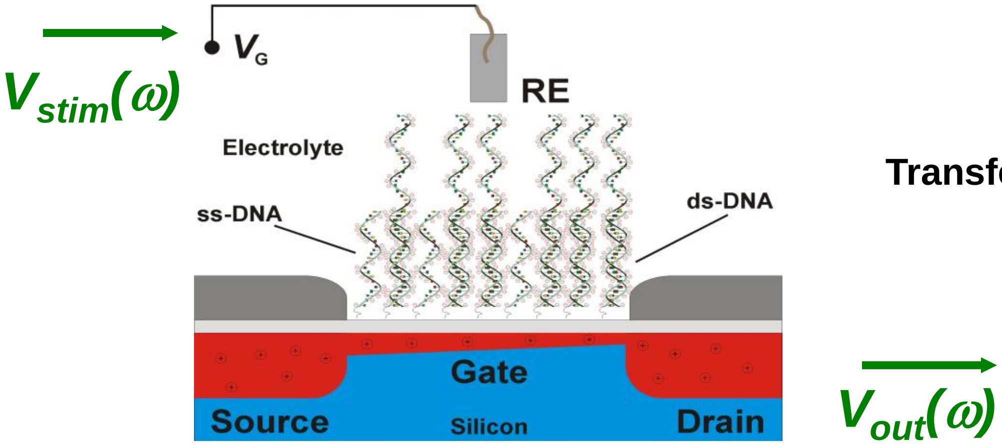
- The gate potential is modulating the inversion channel between drain and source
- Changes in drain-source current are proportional to changes of the gate potential (in the linear region)
- <u>Two signal components:</u>

  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

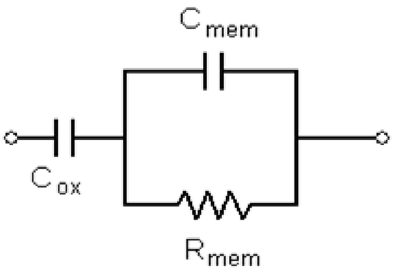
Detection principles: Impedimetric measurements

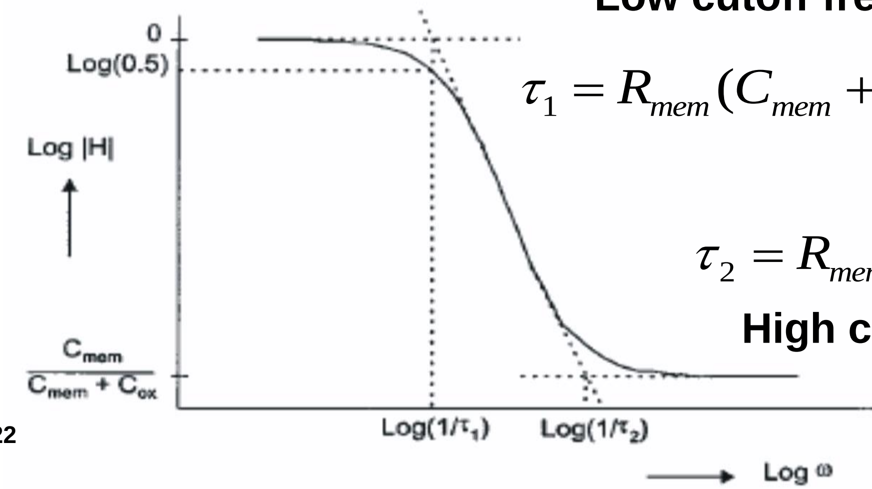
ISFET as impedimetric biosensor – Transistor-Transfer Function (TTF)



Transfer Function:

$$H(\omega) = \frac{V_{out}(\omega)}{V_{stim}(\omega)}$$





Low cutoff frequency

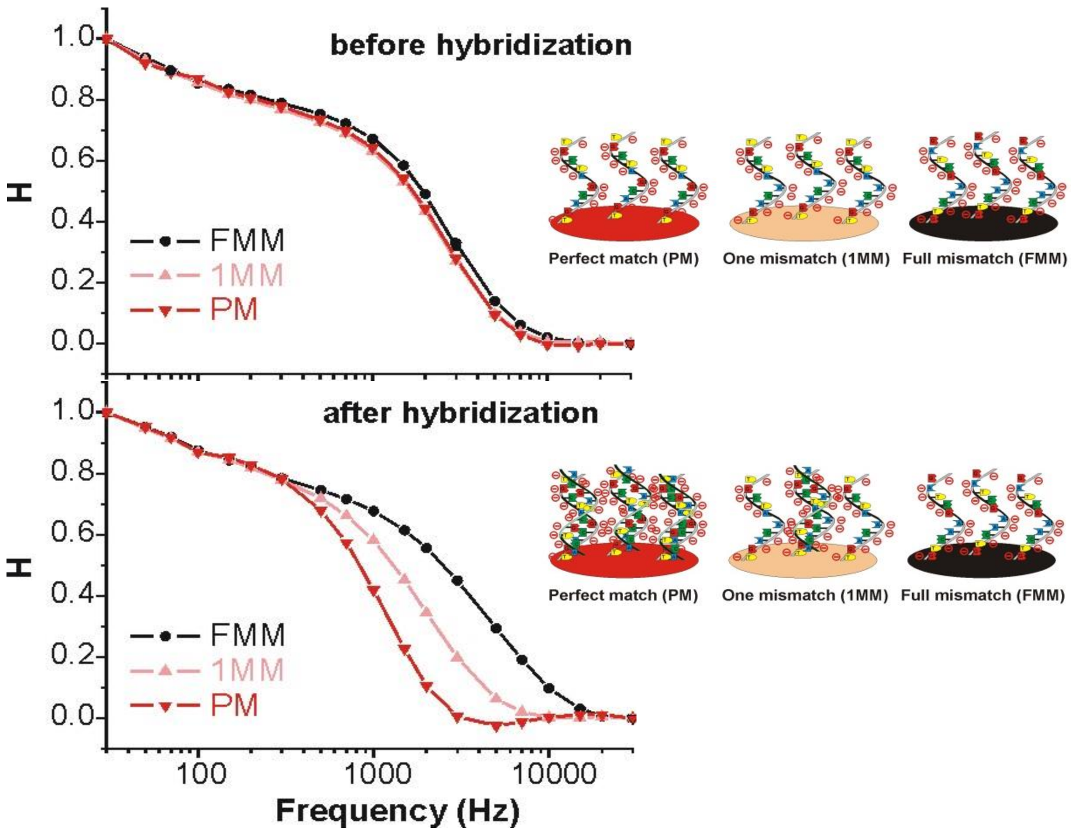
$$\tau_1 = R_{mem}(C_{mem} + C_{ox}) \approx R_{mem} C_{ox}$$

$$\tau_2 = R_{mem} C_{mem}$$

High cutoff frequency

Schasfoort RBM et al., (1989) Sensor Actuator, 18, 119
Kharitonov, AB et al., (2000), Sens. Actuator B-Chem., 70, 222
Antonisse, MMG et al., (2000), Anal. Chem., 72, 343

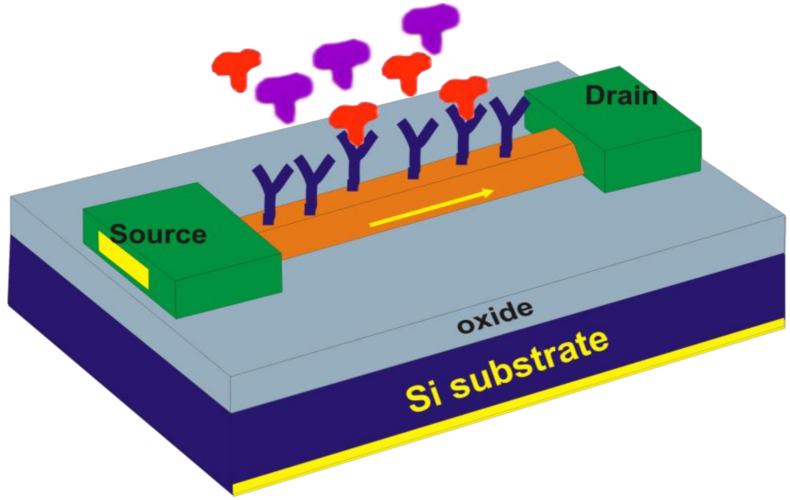
Bio-sensing applications: Electronic DNA-chip with micro-ISFETs

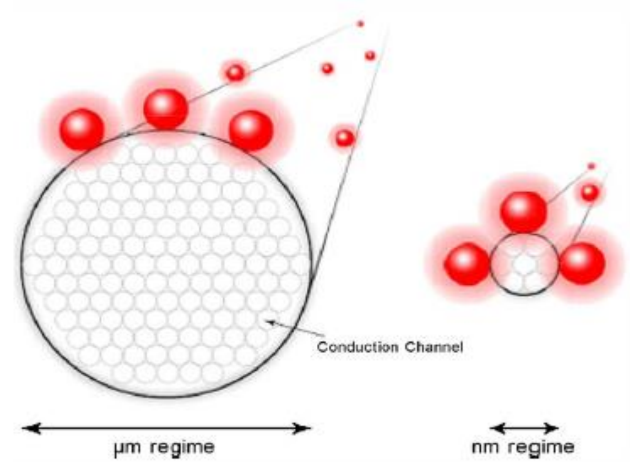


- Novel, lock-in based signal transduction concept
- Reliable recording
- Fast sampling

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

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

Dr. X.-T. Vu, R. Stockmann

Fabrication process of Si-NW arrays

Project start in 2006

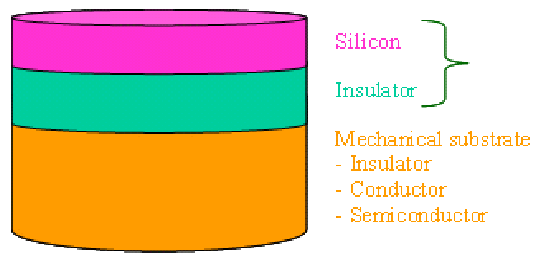
- First trial: Electron beam lithography

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

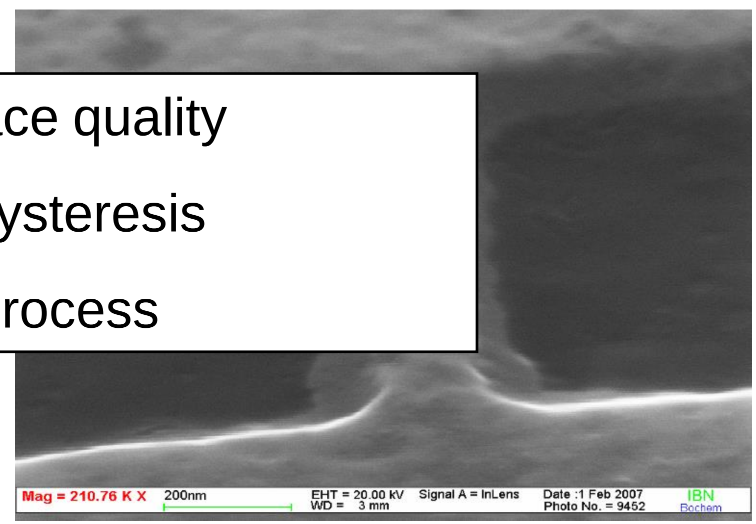
- Electron Beam Writer Leica EBPG 5000 Plus

- High resolution (down to 5nm)

- Maskless patterning



Silicon On Insulator



Bad surface quality

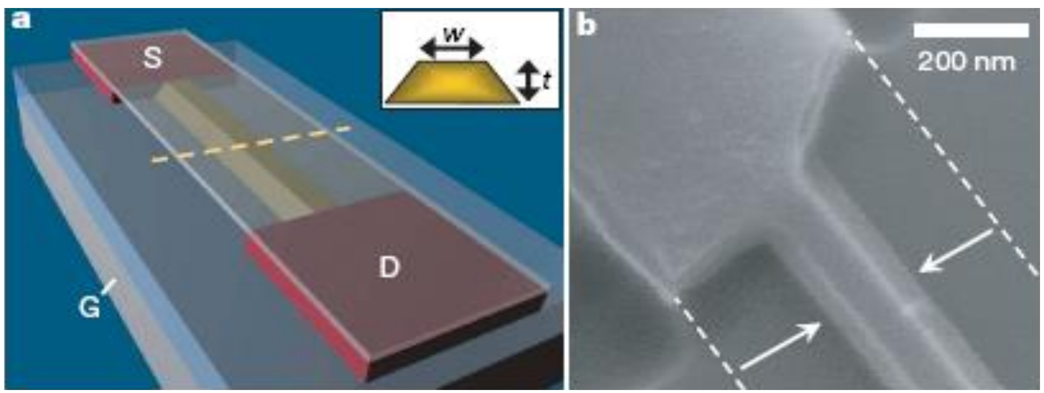
Strong hysteresis

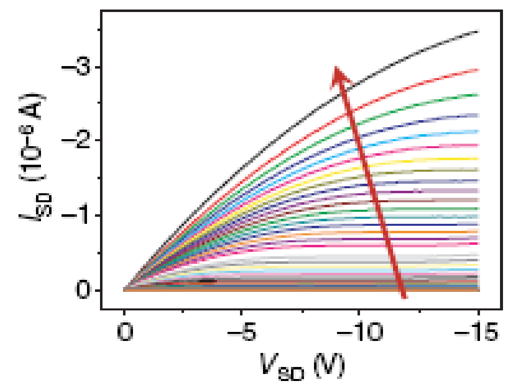
Slow process

Fabrication process of Si-NW arrays

- Silicon nanowire device concept:

- Reed group (Yale)
  - Top-down approach
  - Biomolecular sensing demonstrated recently



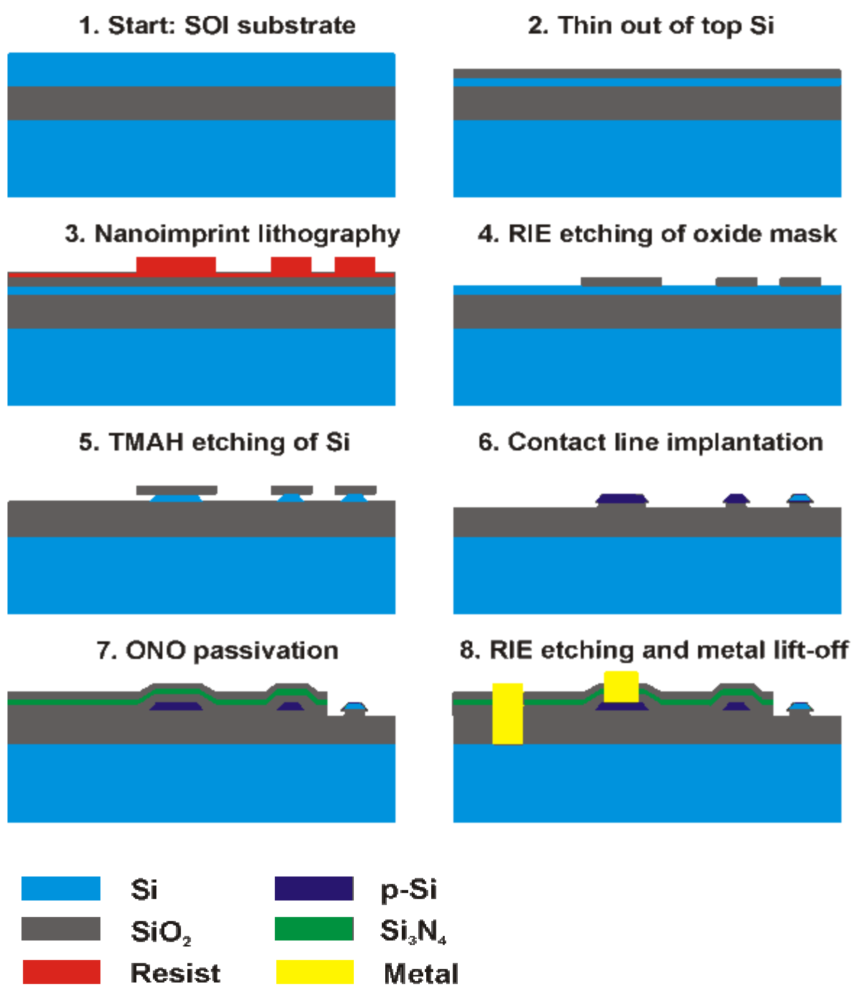


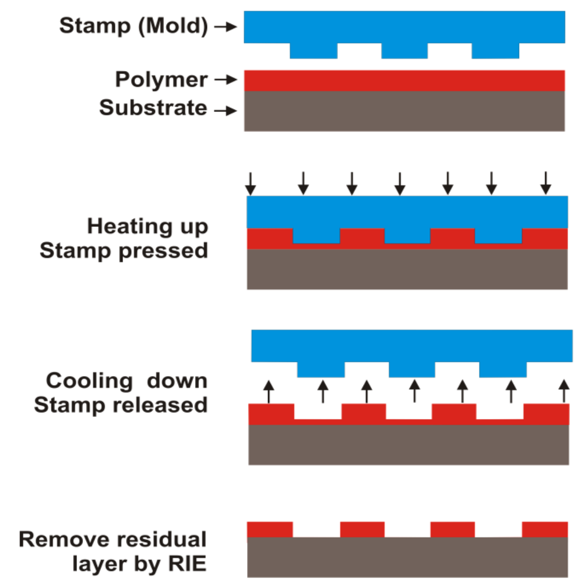
[Source: Stern er al, Nature, February 2007]

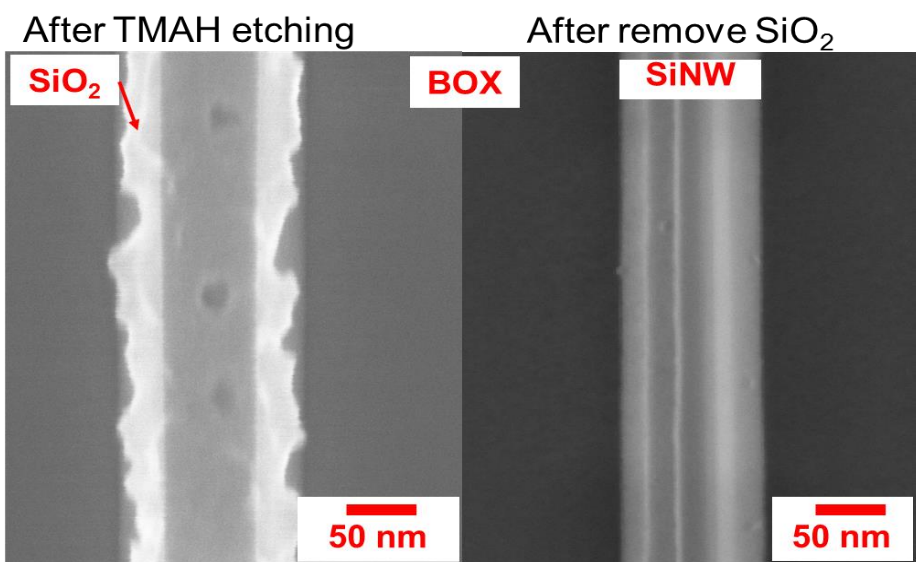
Wet etching process with tetra methyl ammonium hydroxide (TMAH)

Dr. X.-T. Vu, R. Stockmann

Wafer-scale fabrication of SiNW arrays



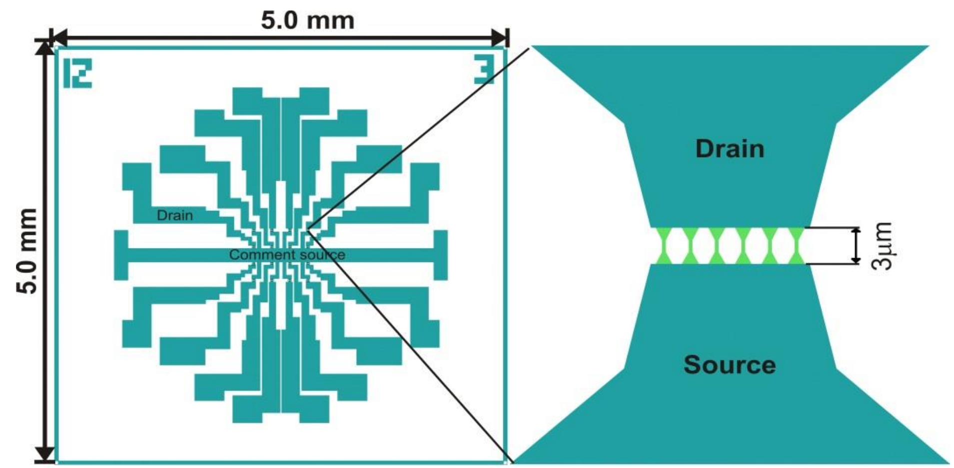




X.T.Vu et al, Physica Status Solidi, 206 (2009) 426
X.T. Vu et al, Sensors and Actuators – B Chemical, 144 (2010) 354

Chip designs

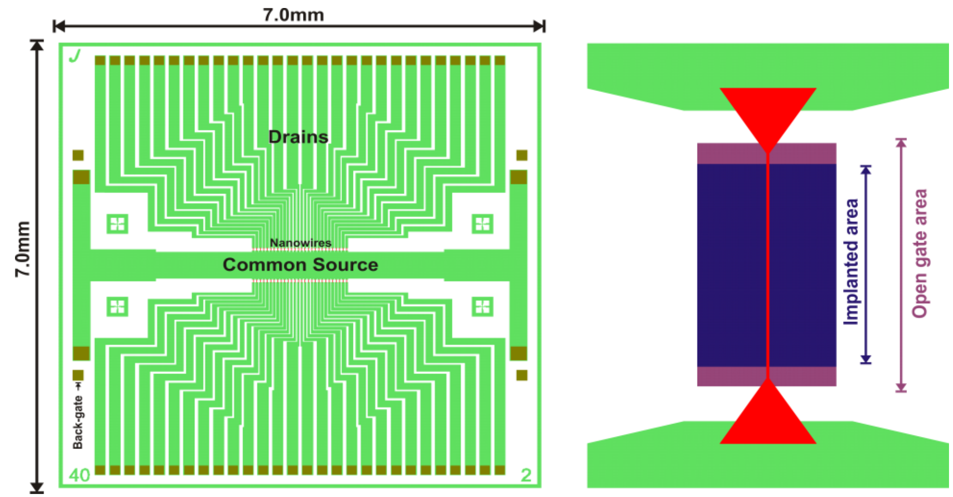
4×4 Arrays
6 wires parallel
Length: 3mm
Width: 100, 200, 500, 1000nm (mask design)
pitch: 200mm



X.T.Vu et al, Physica Status Solidi, 206 (2009) 426
X.T. Vu et al, Sensors and Actuators – B Chemical, 144 (2010) 354

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

Integration with micro-fluidics



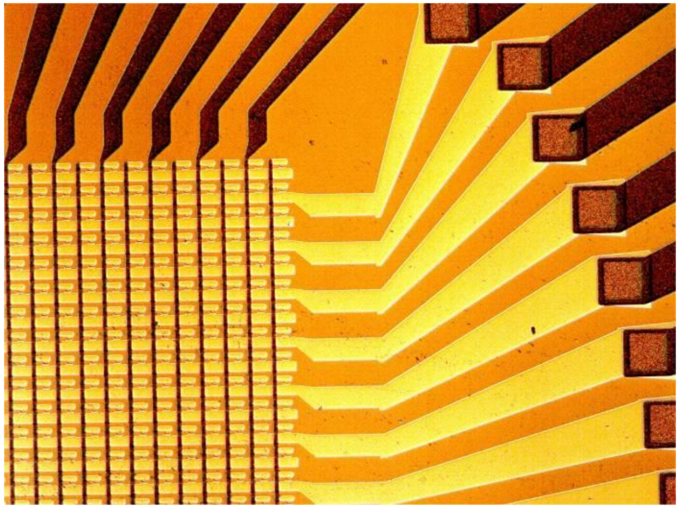
X.T. Vu, et al.,Phys. Status Solidi A-Appl. Mat., 207, 4, (2010) 850-857.

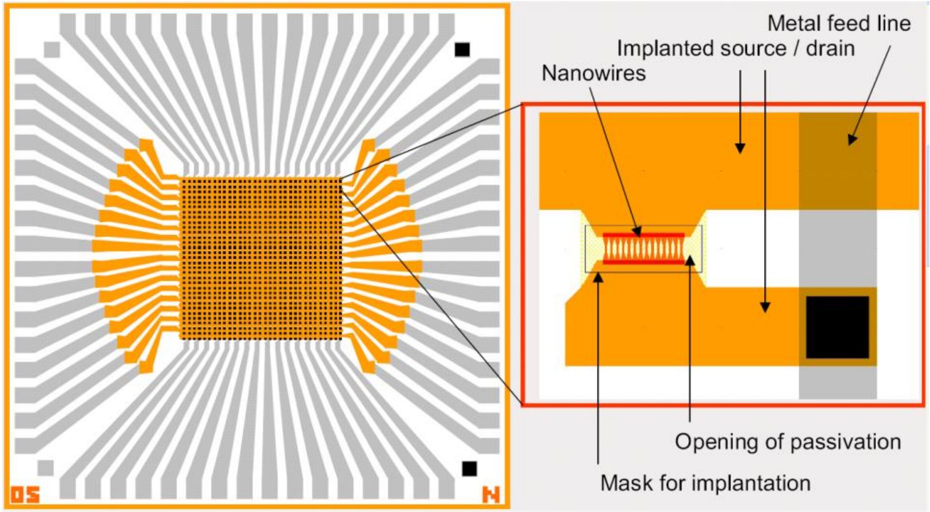
16×16, 32×32 and 128×128 arrays

Dr. X.-T. Vu, R. Stockmann

Fabrication process of Si-NW arrays

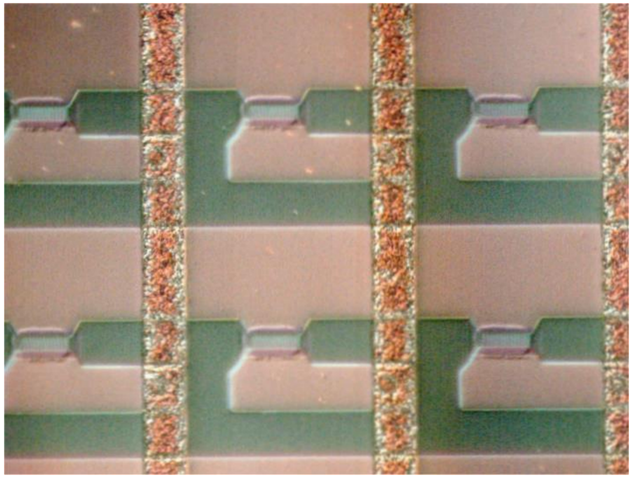
Fabrication of larger Si-NW FET arrays



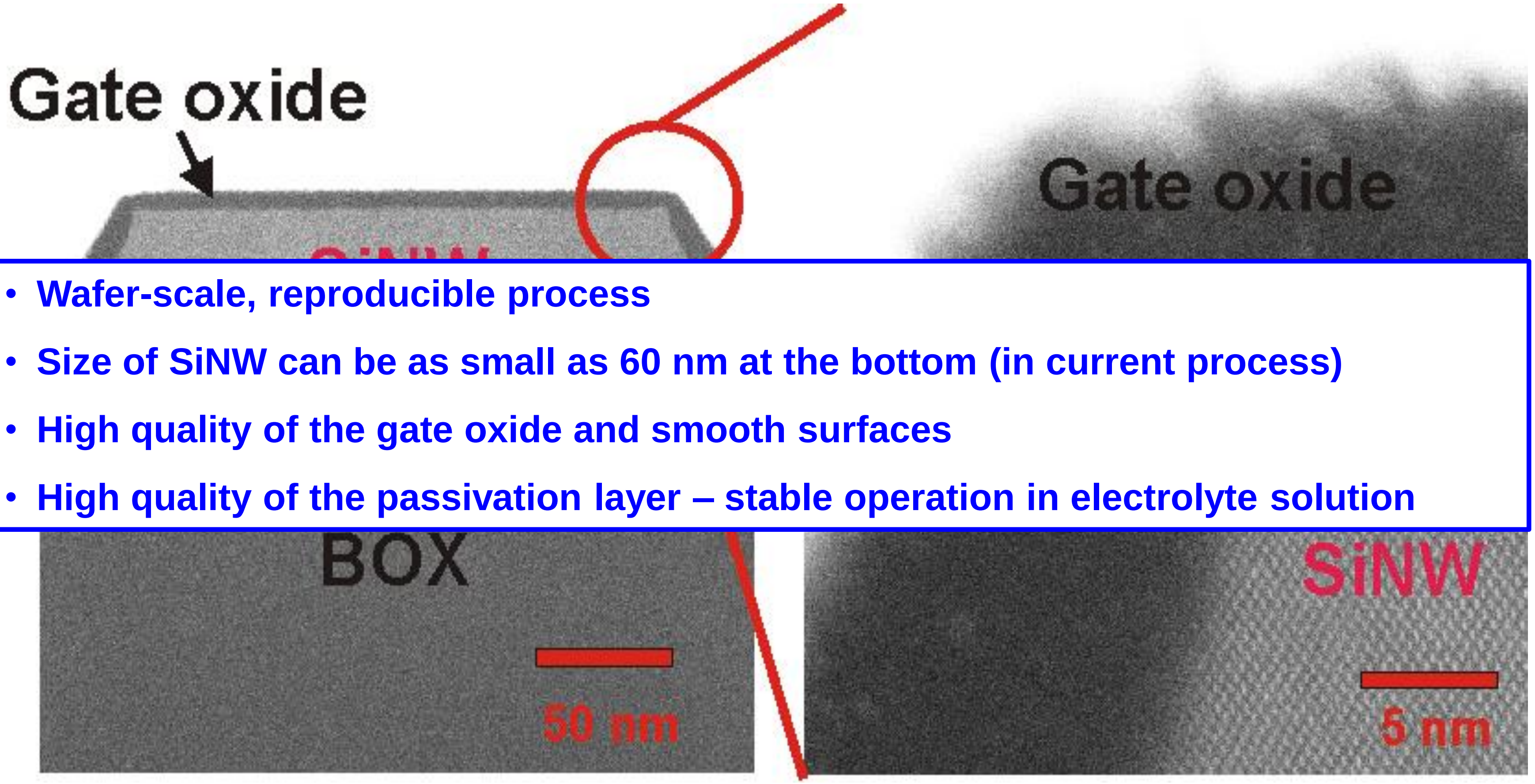


Two designs:
16×16 with 100 µm pitch (256 channels)
32×32 with 50 µm pitch (1024 channels)

- **PECVD passivation was done in Zweibrücken**



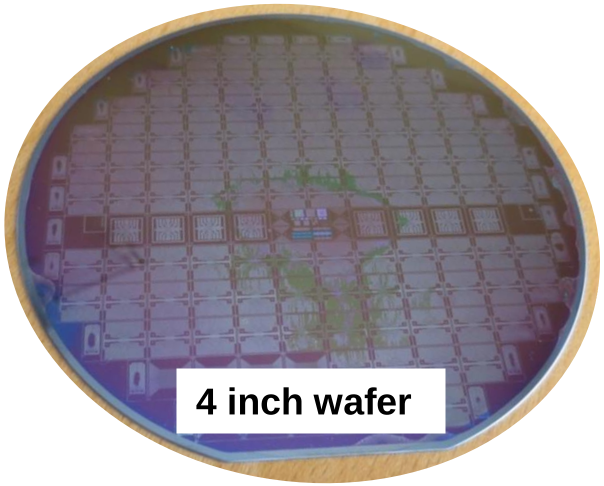
Fabrication process of Si-NW arrays: SiNW devices

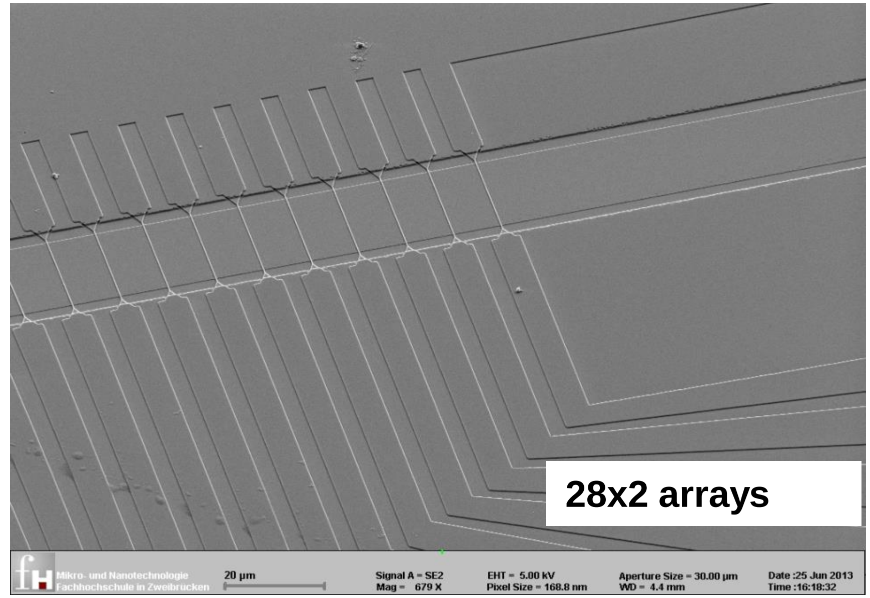


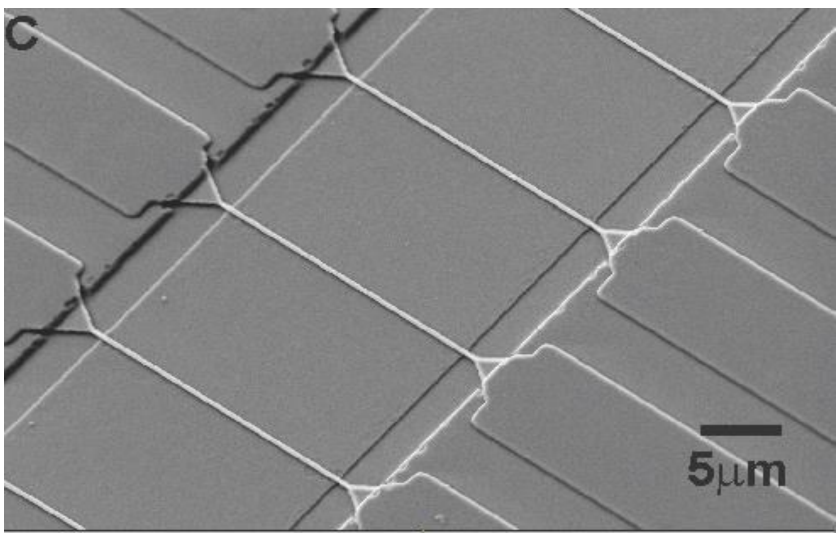
- Wafer-scale, reproducible process
- Size of SiNW can be as small as 60 nm at the bottom (in current process)
- High quality of the gate oxide and smooth surfaces
- High quality of the passivation layer – stable operation in electrolyte solution

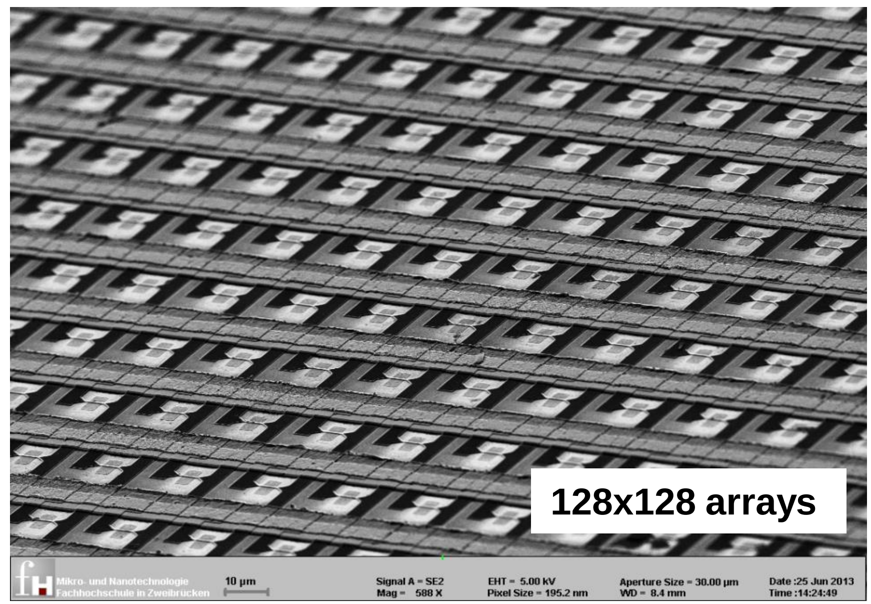
Dr. X.-T. Vu, R. Stockmann

Fabrication process of Si-NW arrays: Wafer-scale processing





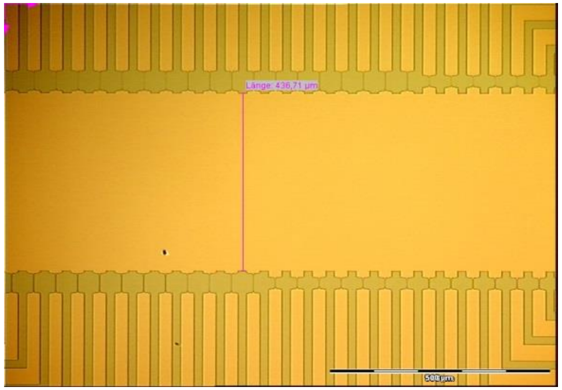
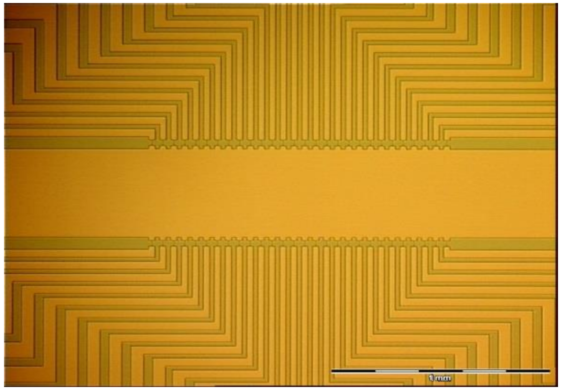
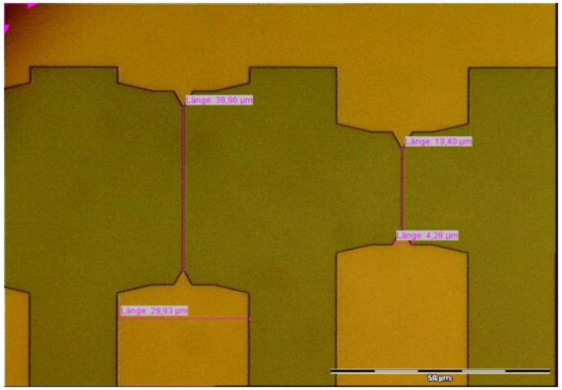




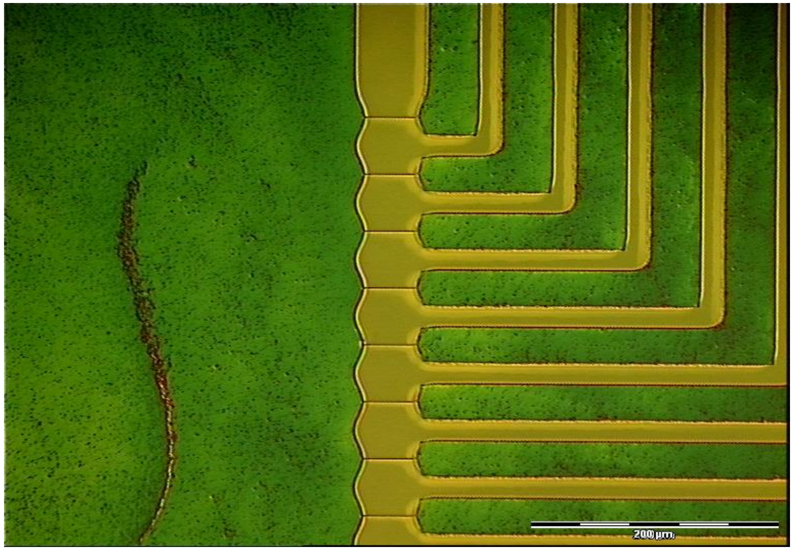
Fachhochschule Kaiserslautern
University of Applied Sciences
Informatik und Mikrosystemtechnik
Zweibrücken

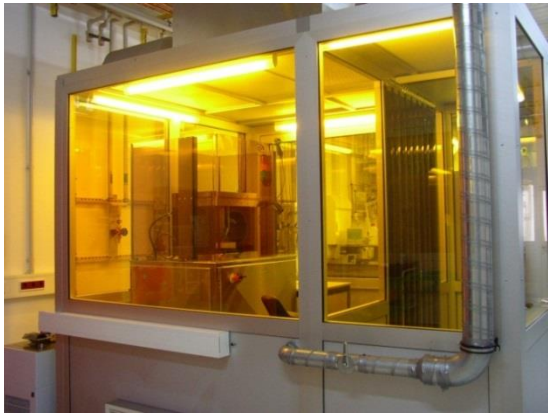
L. Delle, Dr. X.-T. Vu

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

Mold: nanowire structure

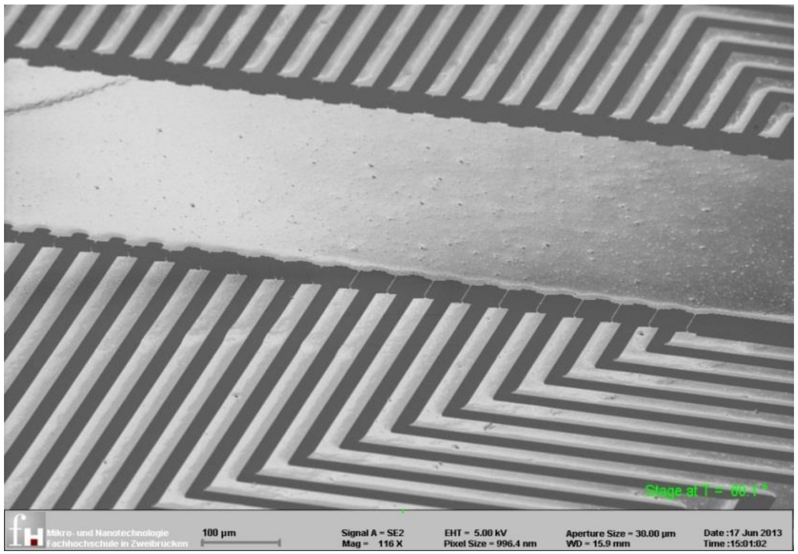




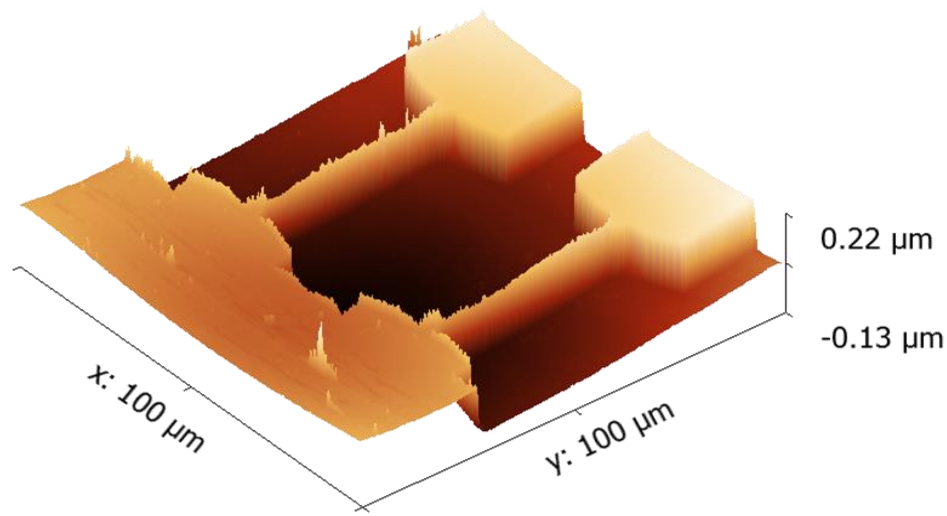
Imprint: nanowire structure in PMMA

L. Delle, Dr. X.-T. Vu

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



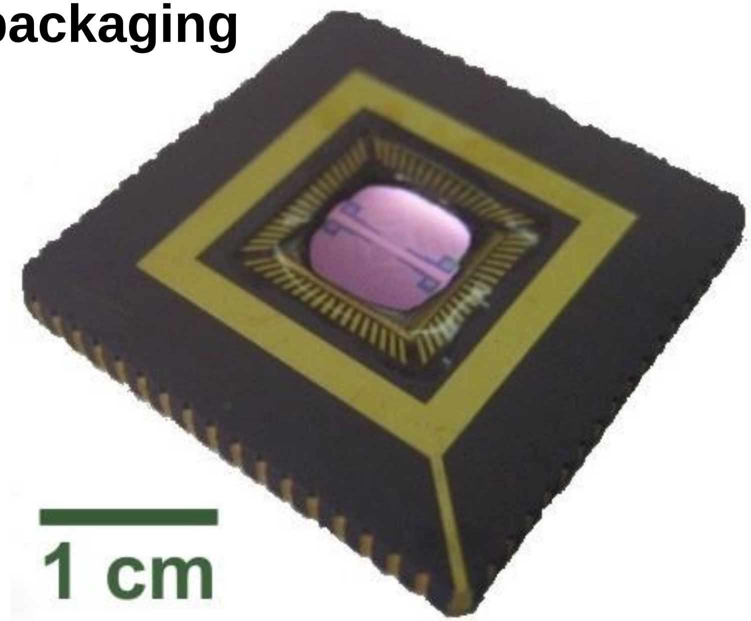
SEM image of nanowire structure etched into SiO_2

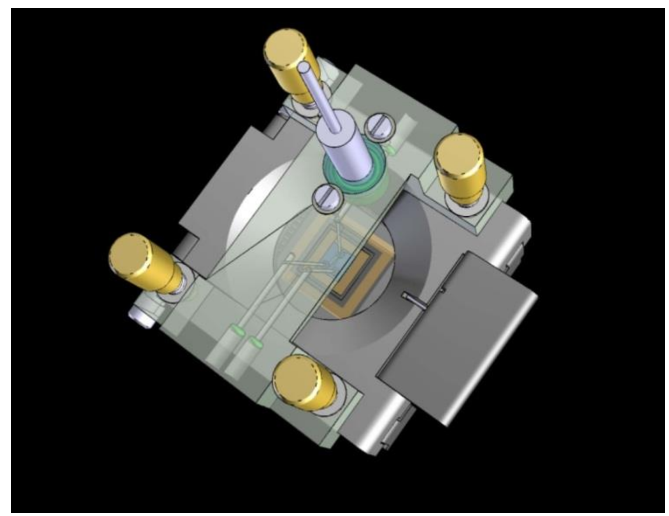


AFM image of PMMA imprinted nanowire structure

Fabrication process of Si-NW arrays: Chip packaging

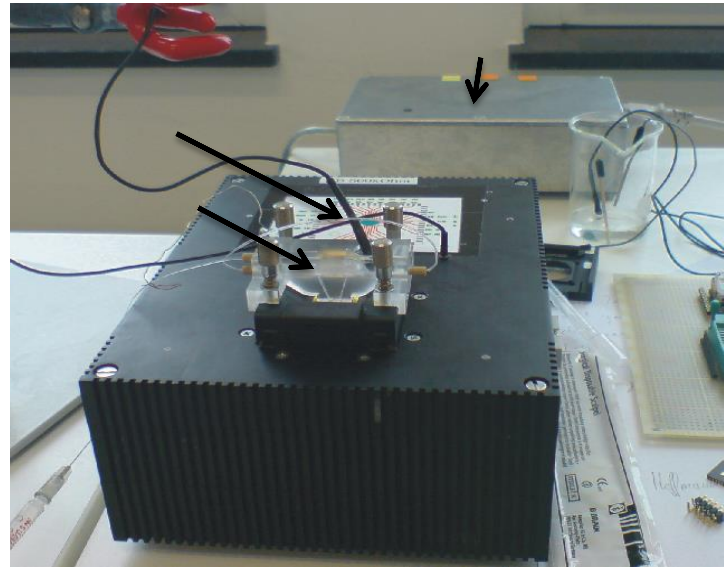
- Wire – bonding
- Contact isolated: Medical epoxy, PDMS
- Can be used directly or integrated with micro-fluidics

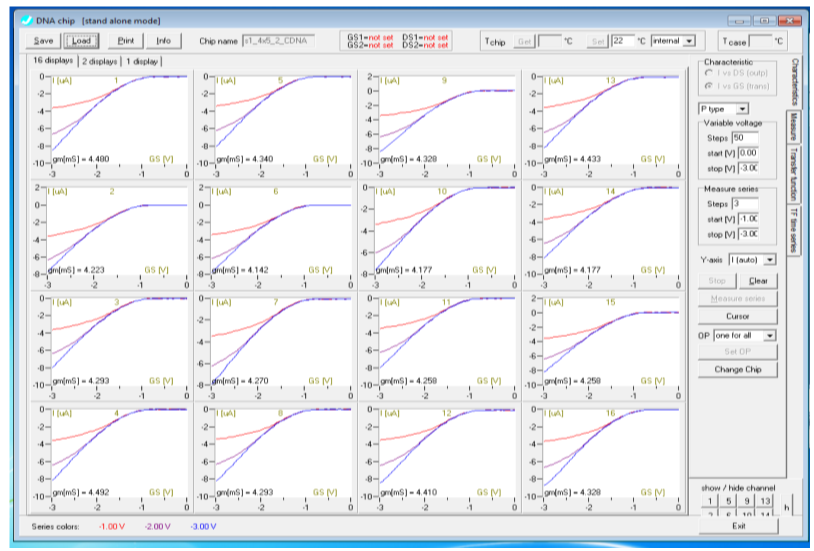




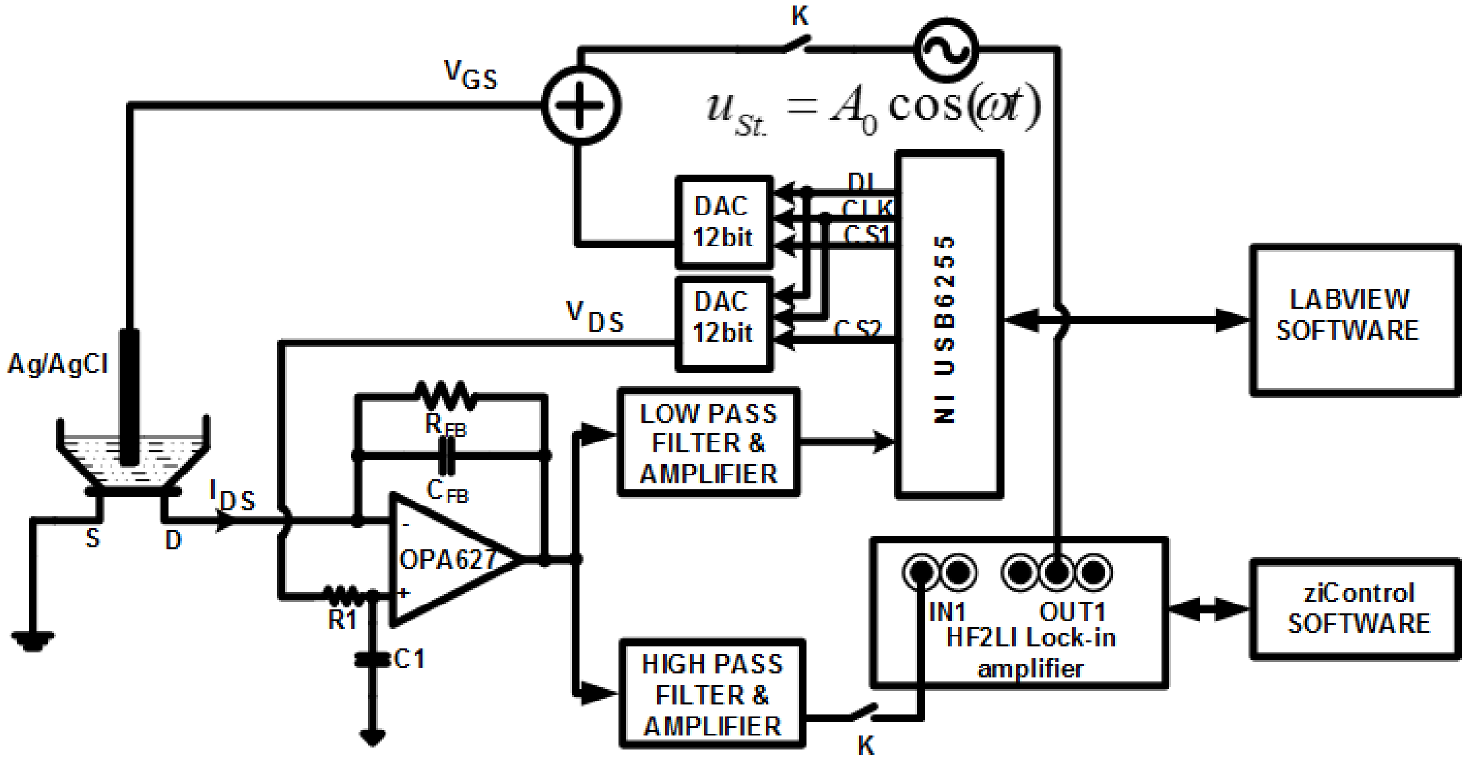
Portable amplifier for the FET arrays

- **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

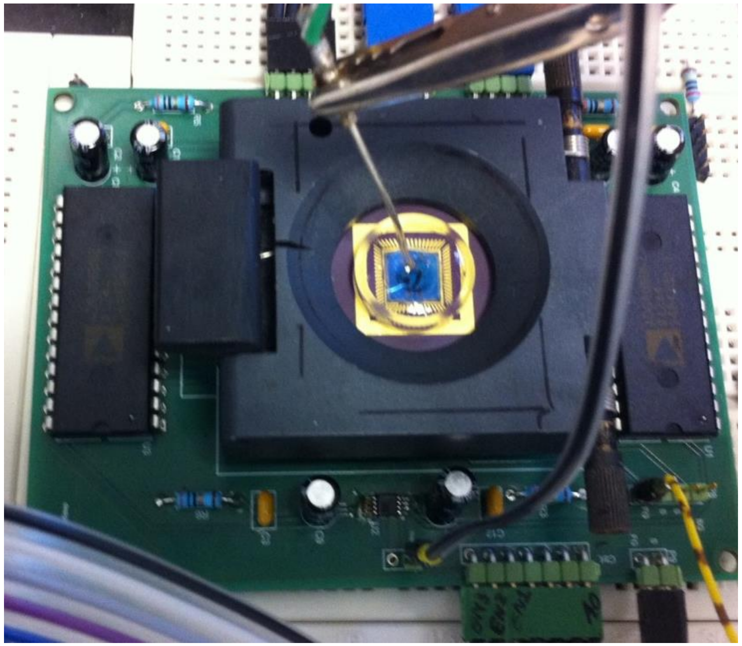


- Here is the design for one channel, different channels are currently addressed by switching
- The system is flexible for different ranges of drain-source currents
- Suitable for both characterization and impedimetric measurement of FET devices

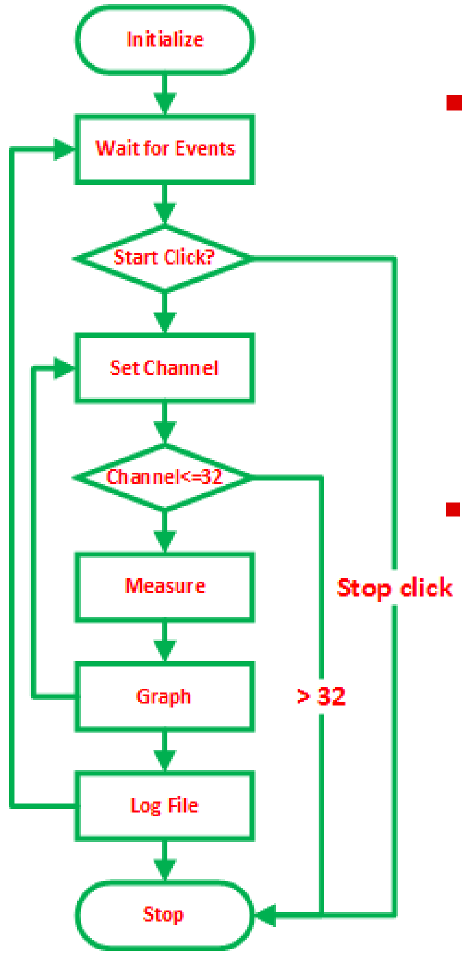
T. C. Nguyen et al., Physica Status Solidi (a), 210, 5, 870–876 (2013).

NEW amplifier system

LabVIEW programming



Headstage setup

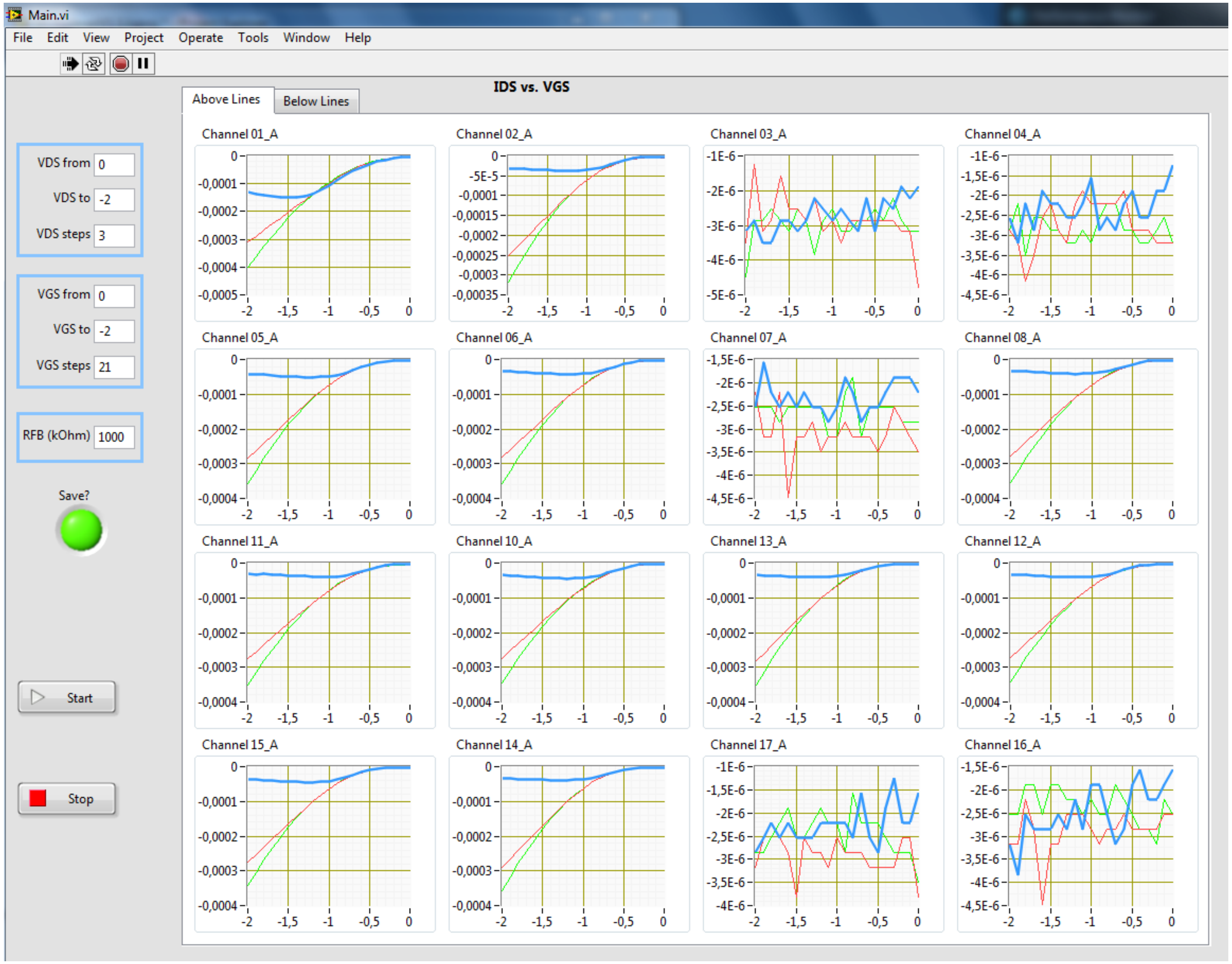


- State machine programming:
  - Program is divided into different states
  - Easier to read
  - Easier to manage
  - Easier to scale
- Stand-alone application: no LabVIEW development tool is needed

At the moment still single channel

T. C. Nguyen et al., Physica Status Solidi (a), 210, 5, 870–876 (2013).

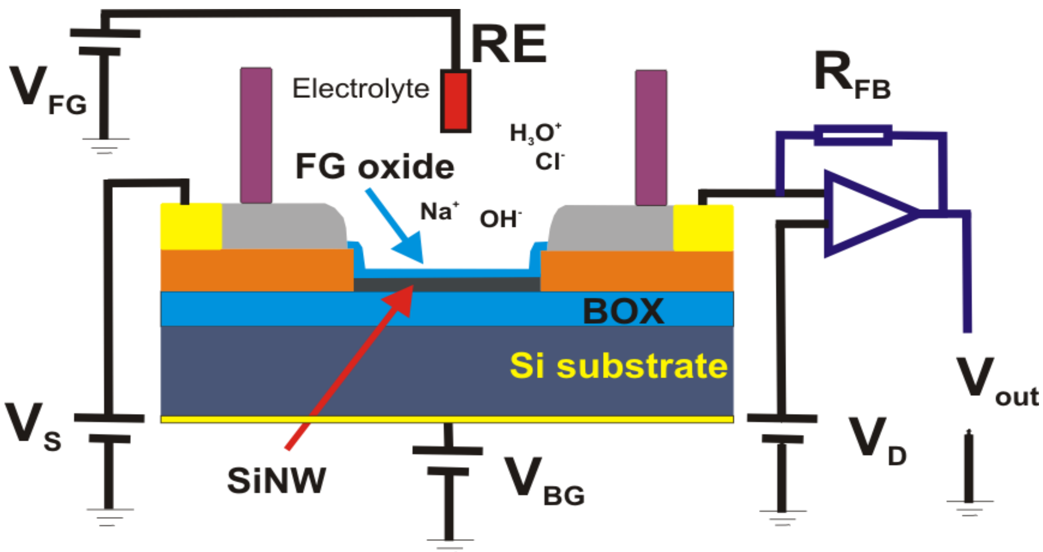
NEW labview-based readout software



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

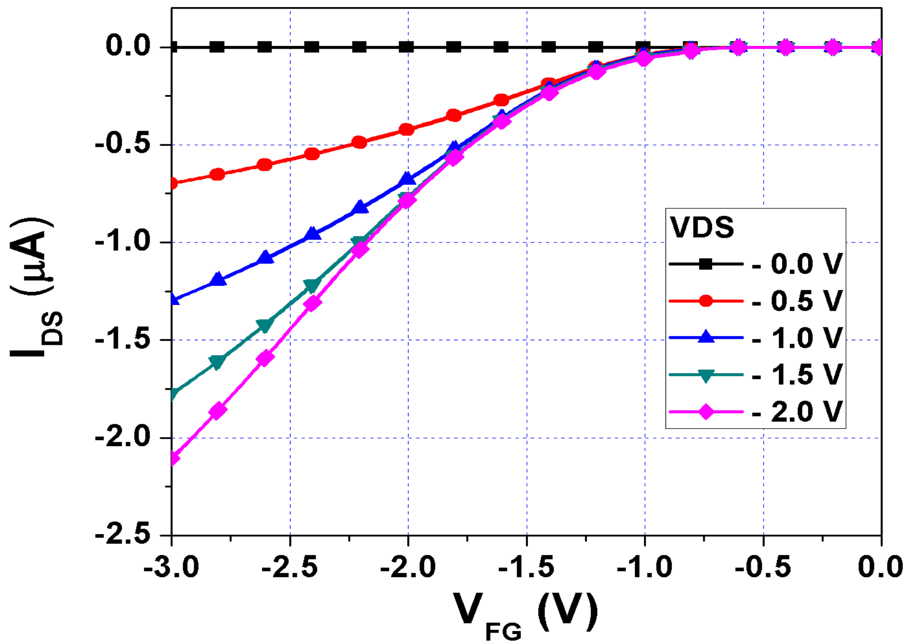
Electrical characteristics

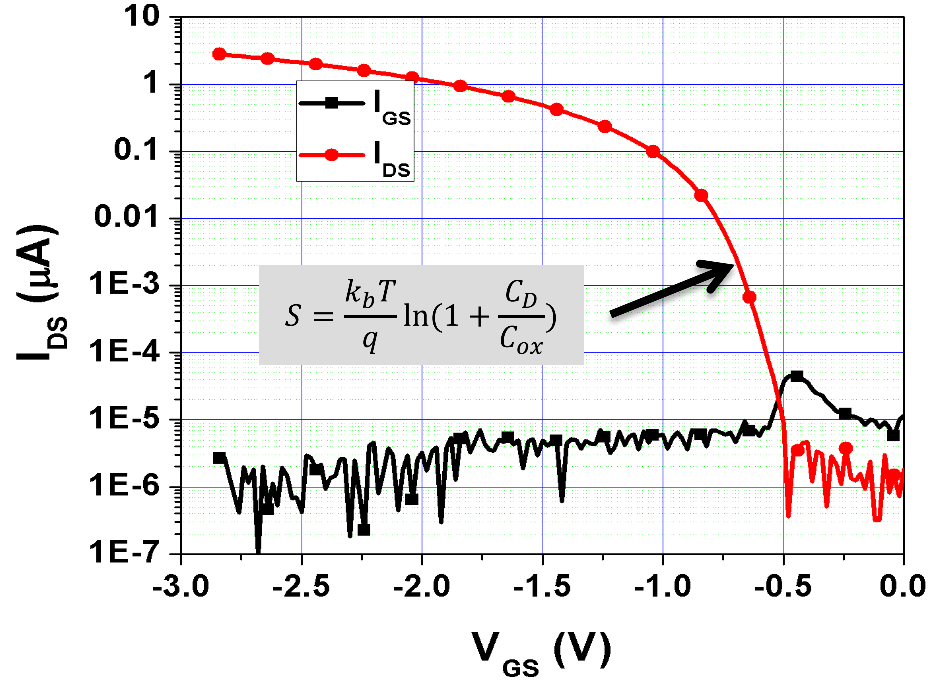
	Implanted CL	Non-implanted CL
Back-gate control (in dry and wet environment)	p-type only unstable operation	p- and n-type unstable operation
Front-gate control (in wet environment)	p-type only stable operation	n-type only stable operation

- 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: $I_{DS}(V_{GS})$

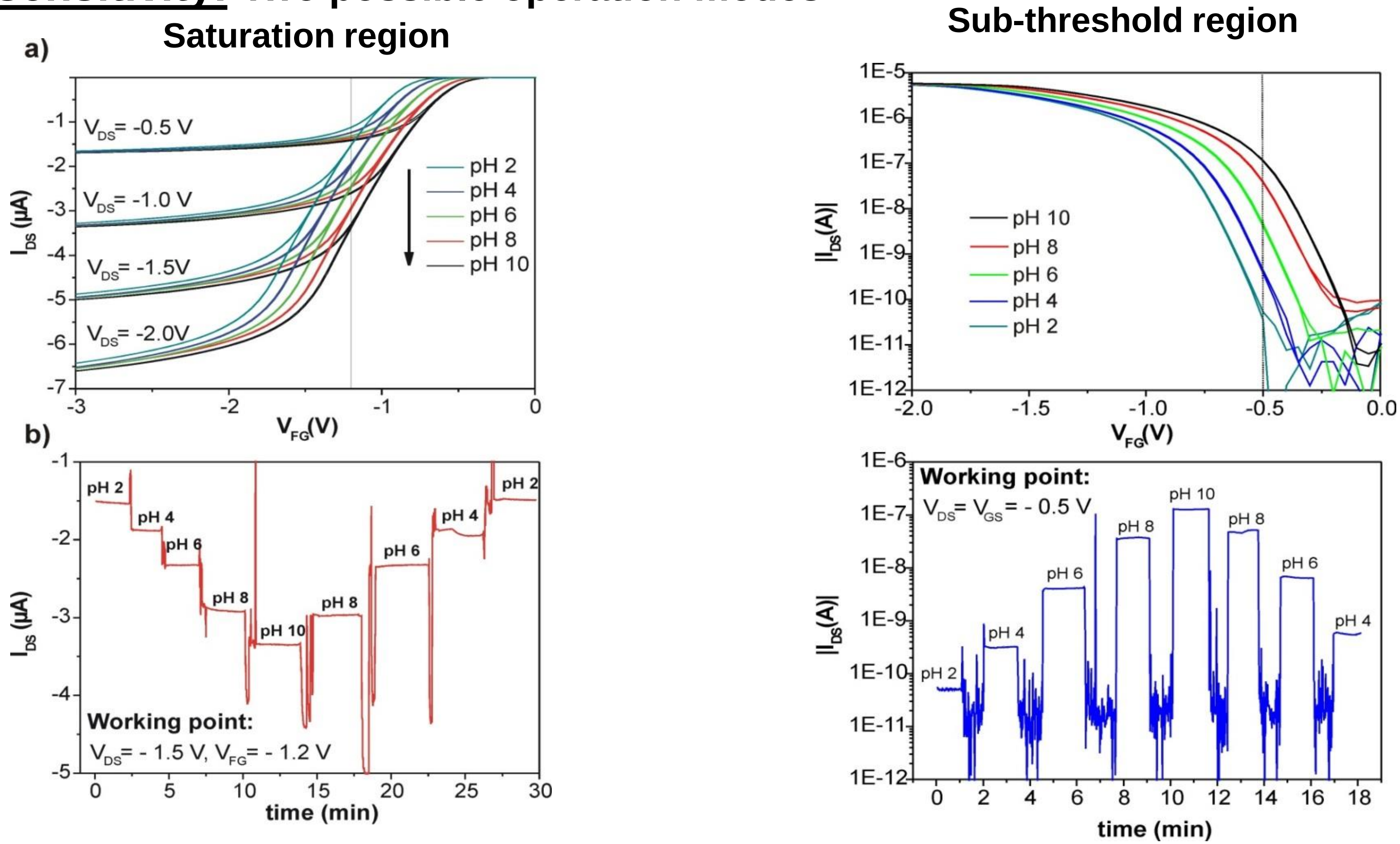




- **P- enhancement and long-channel transistor**
- **V_{TH} = - 0.8 V at pH 7**
- **$g_{m,\,max}$ (at V_{DS}=-2V): 0.5 μS to 10 μS**
- **I_{on}/I_{off} =10^5-10^6**
- **S = 80 - 110 mV/decade, size dependent**

Electrochemical characterization

pH sensitivity: Two possible operation modes

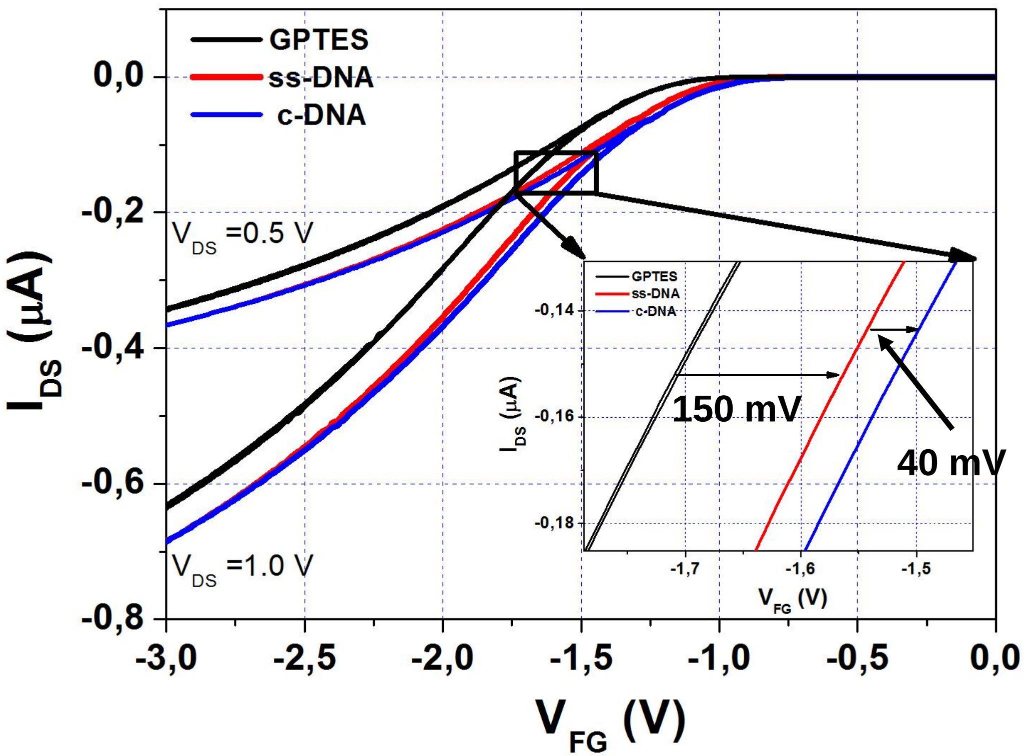


X.T. Vu, et al.,Phys. Status Solidi A-Appl. Mat., 206, 3, (2009) 426-434.

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: ΔV_G up to 150 mV
Hybridization: ΔV_G up to 40 mV

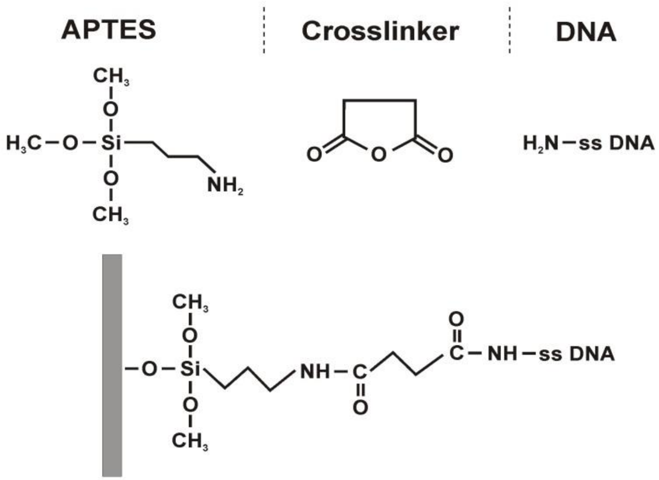
GPTES ssDNA dsDNA

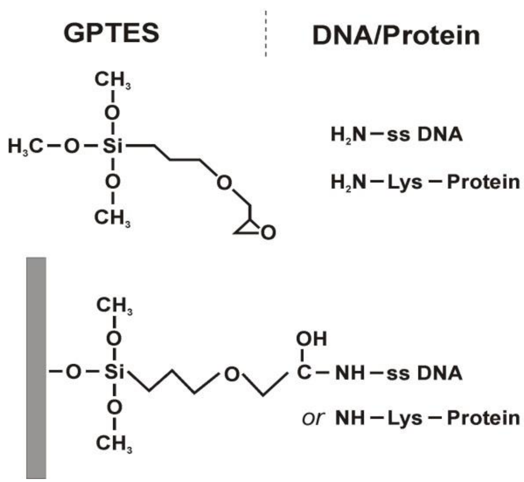
ISFET

Immobilization: ΔV_G = 1-10 mV
Hybridization: ΔV_G = 1-4 mV
Han et al. 2006

SiNW sensor is much more sensitive than our microscale ISFETs

Assay 1: Covalent attachment of DNA probes

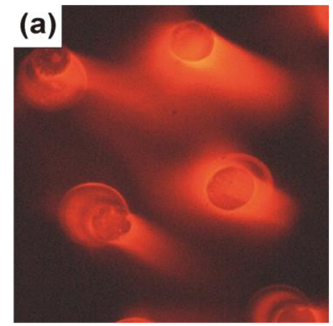




Protocol (a)*:

- Silanization with 3-aminopropyltriethoxysilane
- Usage of succinic anhydride as cross linker
- Covalent attachment of amino-functionalized DNA

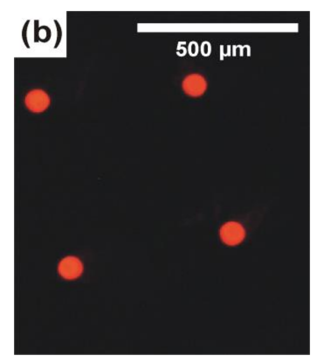
(a)



Protocol (b)**:

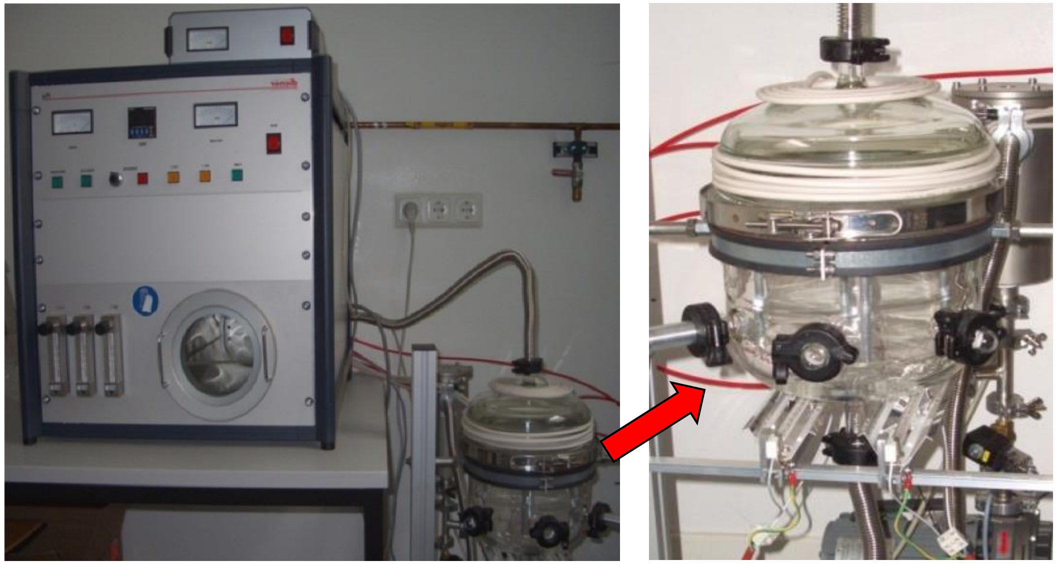
- Silanization with 3-glycidoxypropyltrimethoxysilane
- Direct, covalent attachment of amino-functionalized DNA and of proteins

(b)



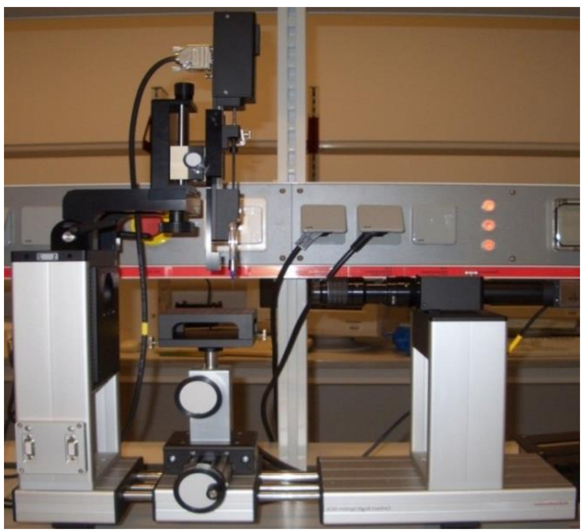
** Han et al., 2006; Ingebrandt and Offenhäusser, 2006.*
***Ghosh-Moulick et al., 2009.*

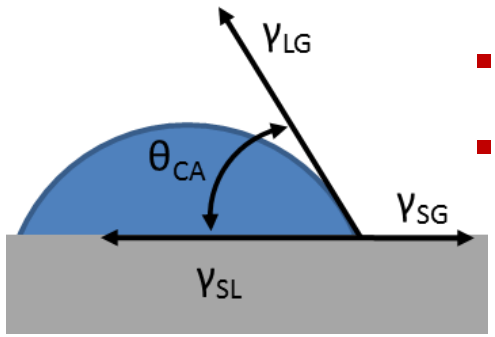
Assay 1: Surface modification - silanization



Gas phase silanization

- **Homogenous monolayers**
- **Encapsulated material not damaged**



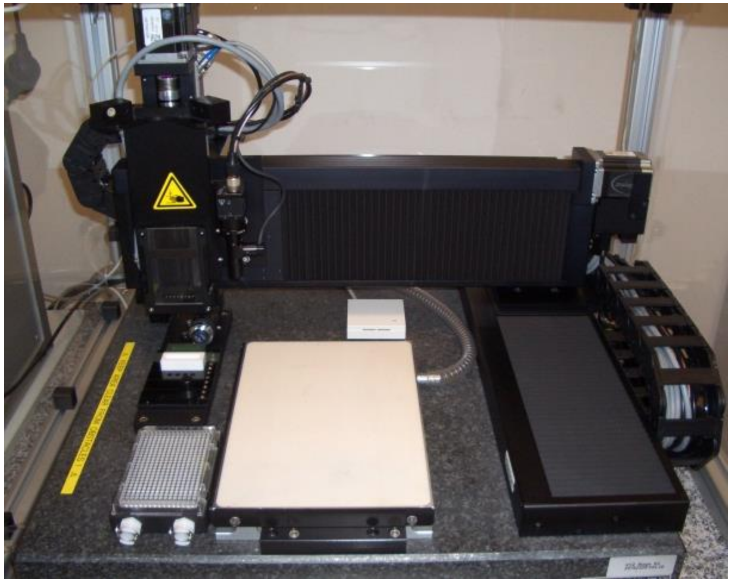


Contact angle measurement

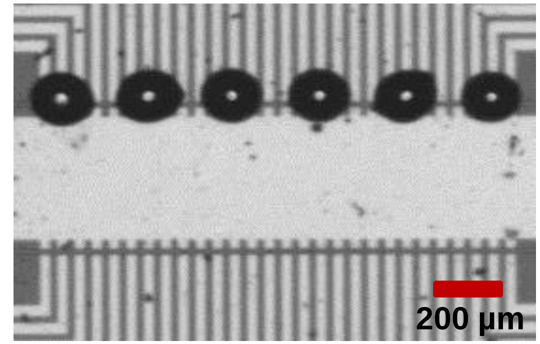
- **Quantify wettability**
- **Qualify silanization:**
  - **Did silanization work?**
  - **Is layer homogeneous?**

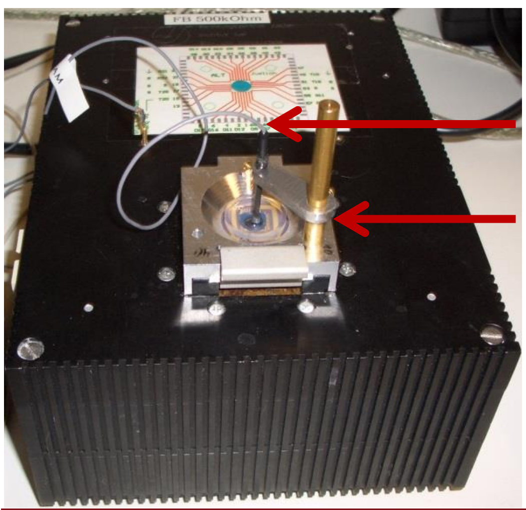
M. Schwartz, Dr. X.-T. Vu

Assay 1: DNA microspotting & electrical readout



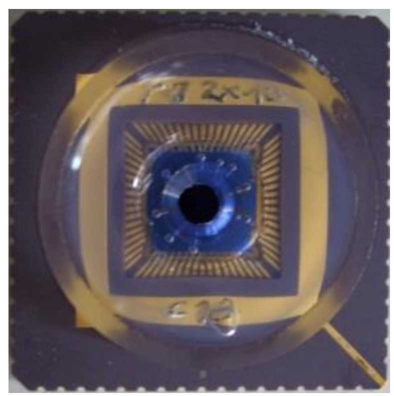
- Site-specific spotting of different capture molecules
- Differential read-out possible
- Defined distances to avoid cross-contaminations

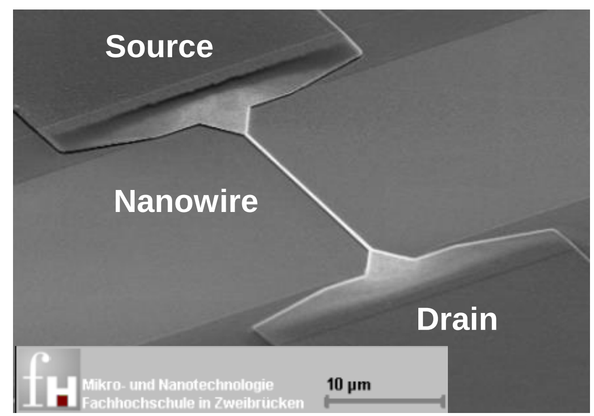




reference electrode

electrode holder

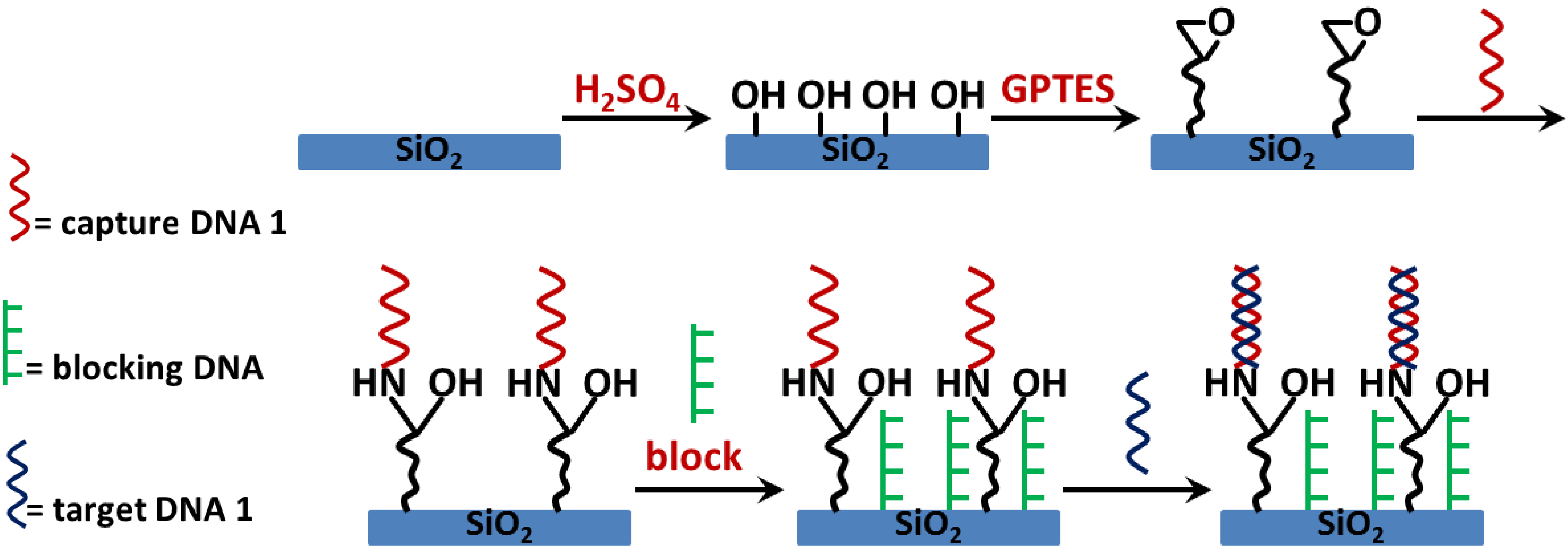




Assay 1: Surface modification steps - overview

3-Glycidoxypropyltrimethoxysilane (GPTES)

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

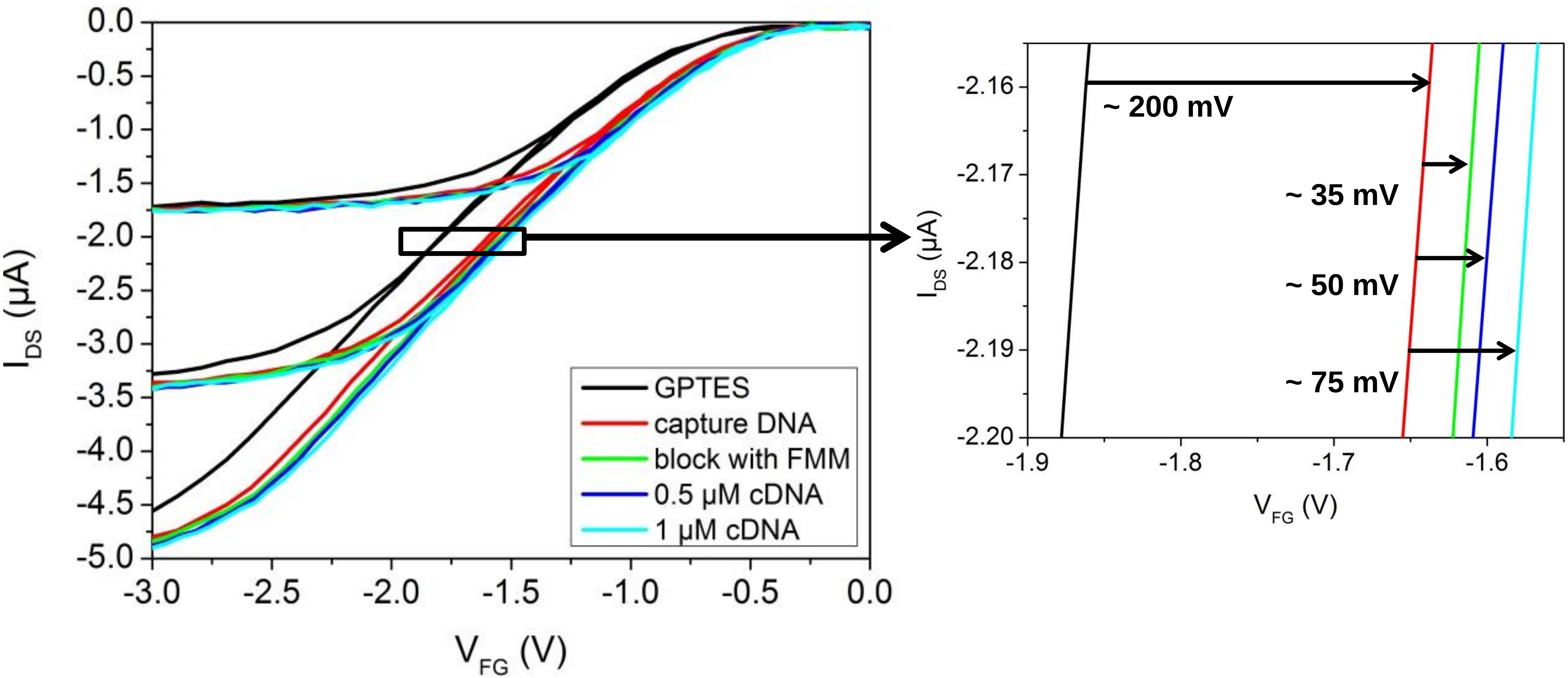


Assay 1: DNA detection – dc readout

Results unpublished

Applied concentrations:

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

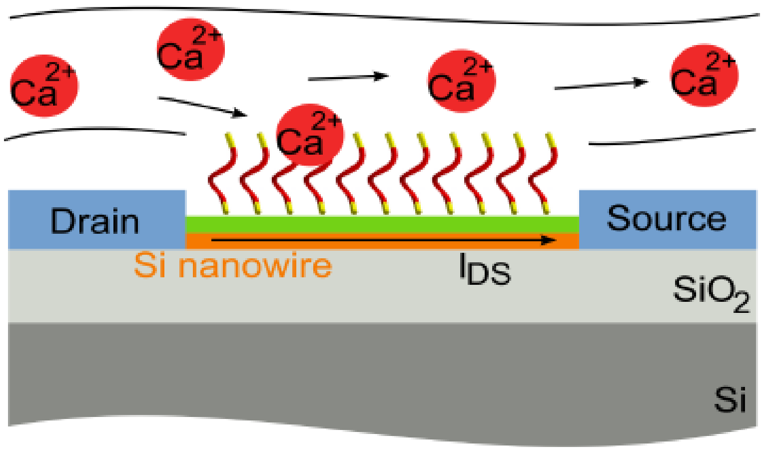


V_{TH} shifted to the right due to binding of negatively charged DNA molecules

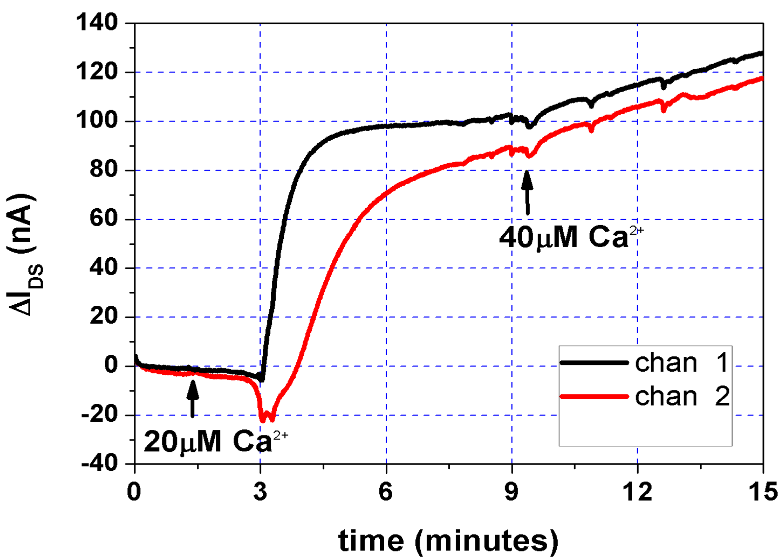
Bioassays
Assay 2: Ca^{2+} ions – peptide interaction

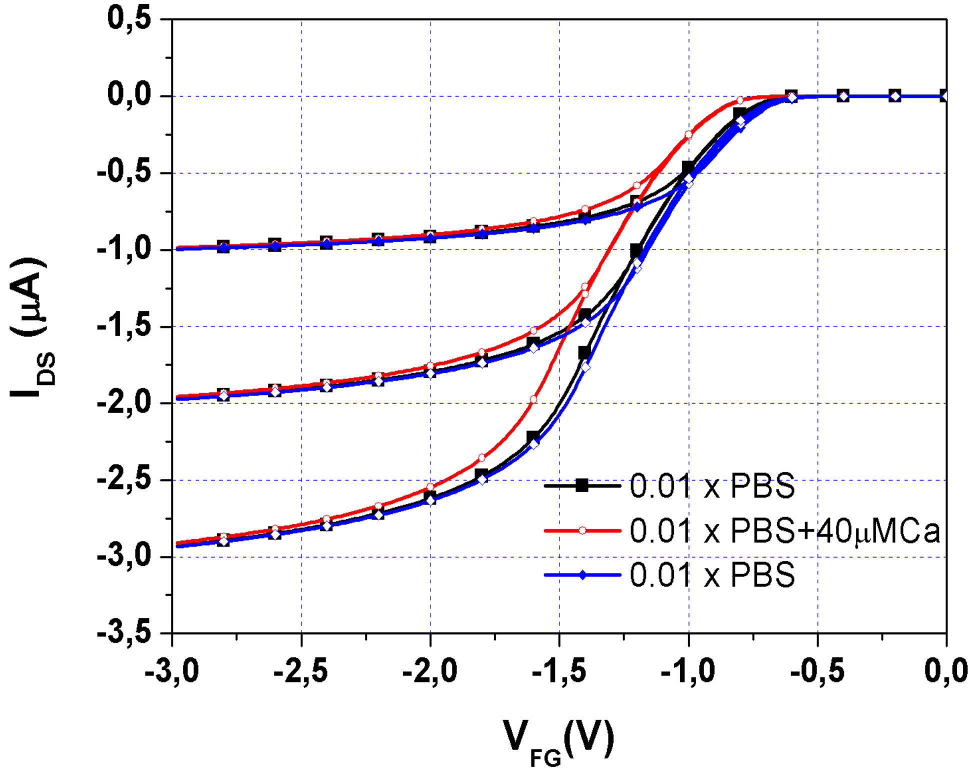
M. Hitzbleck, Dr. X.-T. Vu

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



M. Hitzbleck et al. Submitted to Langmuir

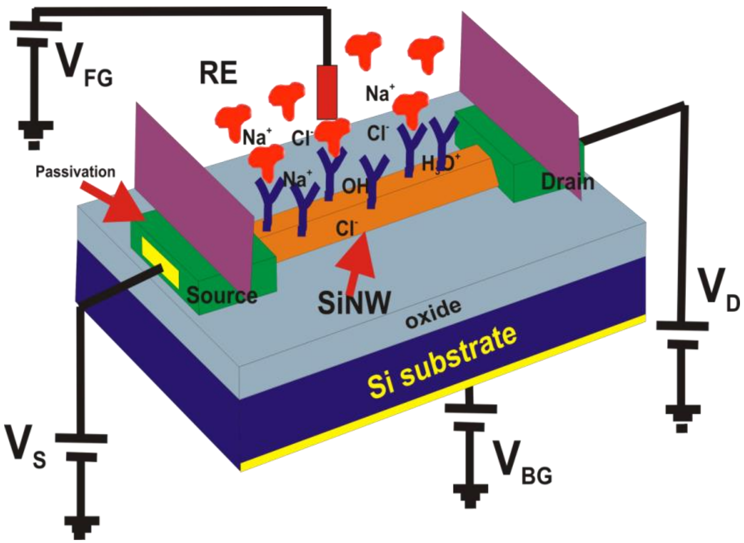


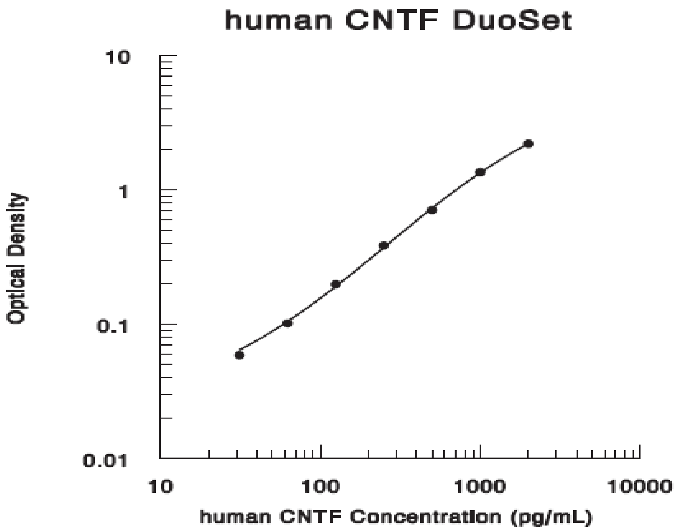


M. Hitzbleck et al., Physica Status Solidi (a), 210, 5, 1030–1037 (2013).

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

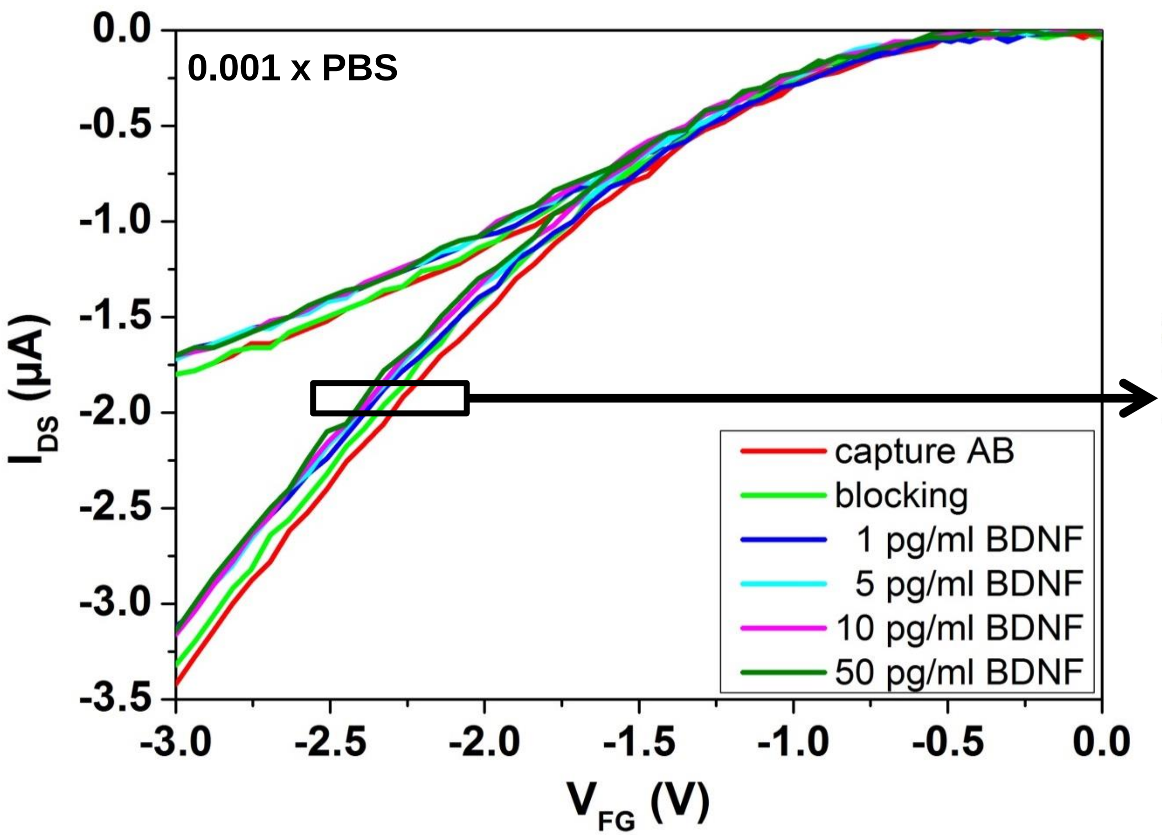
***CNTF: Ciliary neurotrophic factor**

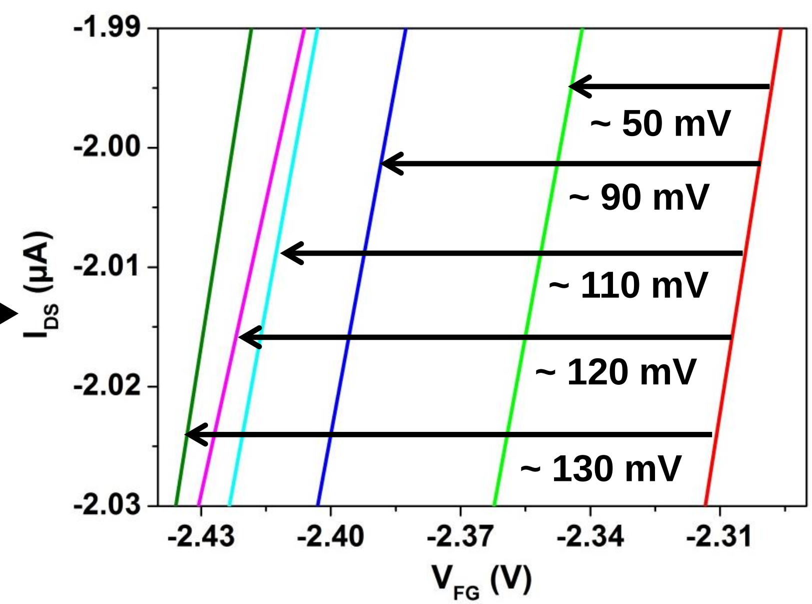
M. Schwartz, Dr. X.-T. Vu

Assay 3: Antibody-antigen – dc readout

Results unpublished

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



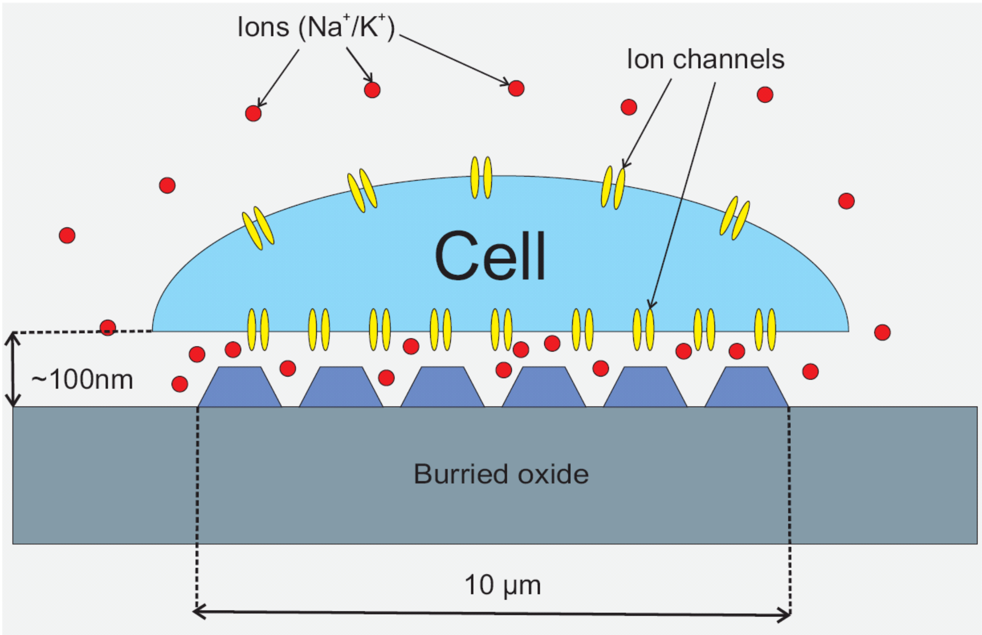


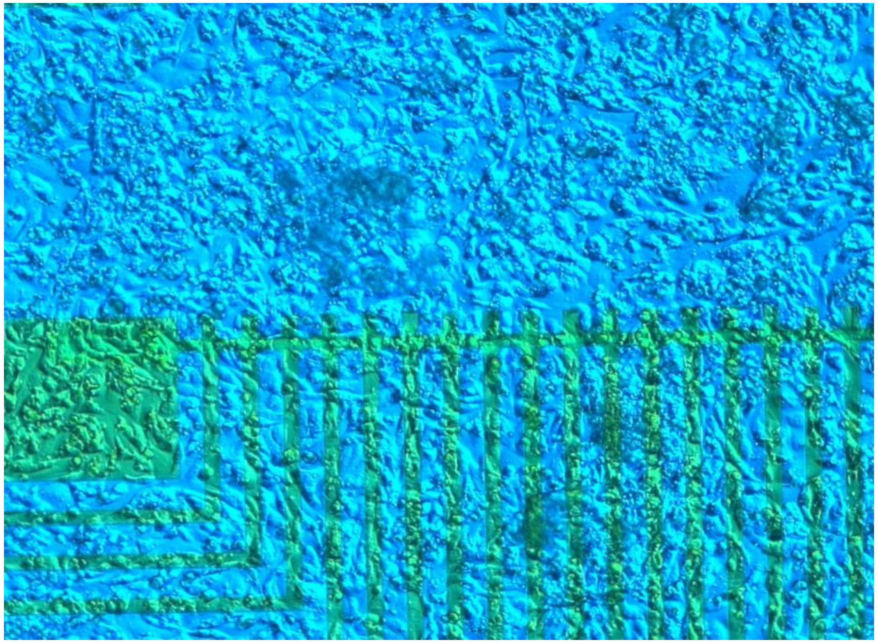
V_{TH} shifted to the left

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





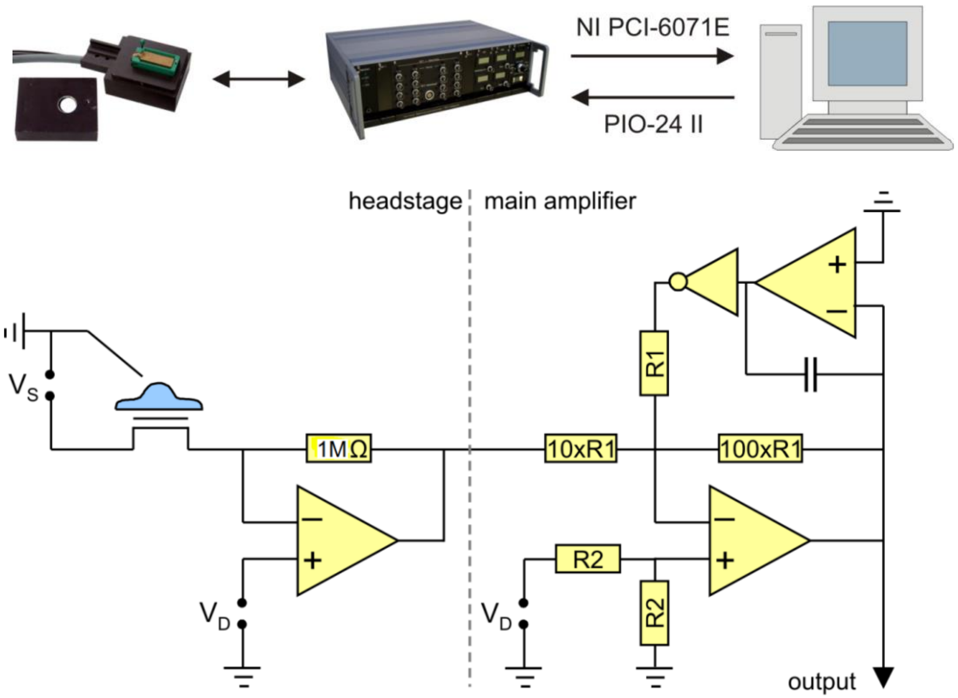
Rat cardiac myocytes at 5 DIV

Once a confluent layer is formed cells are spontaneously beating.

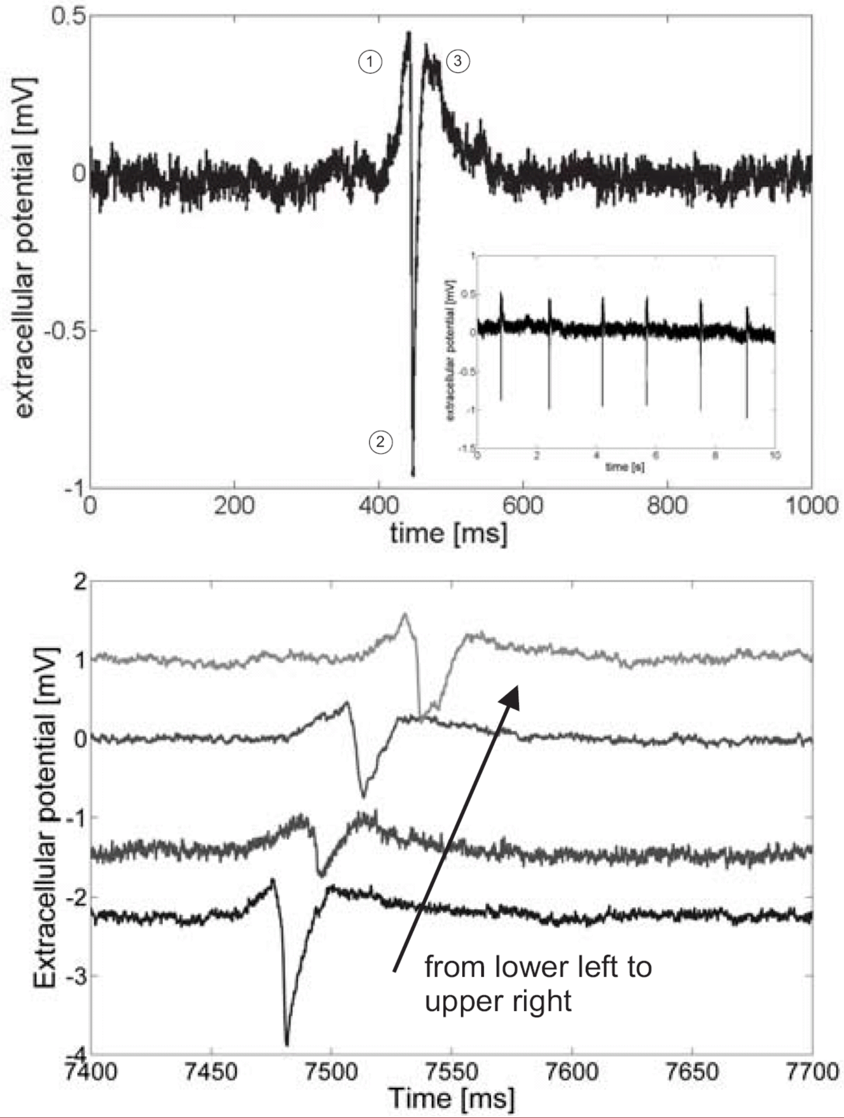
Signals can be site-selectively recorded by the NW-FET array.

Assay 4: Recording of extracellular action potentials

16-channel recording of action potentials



HL1 - cell line



Signals show a similar shape as compared to ISFETs

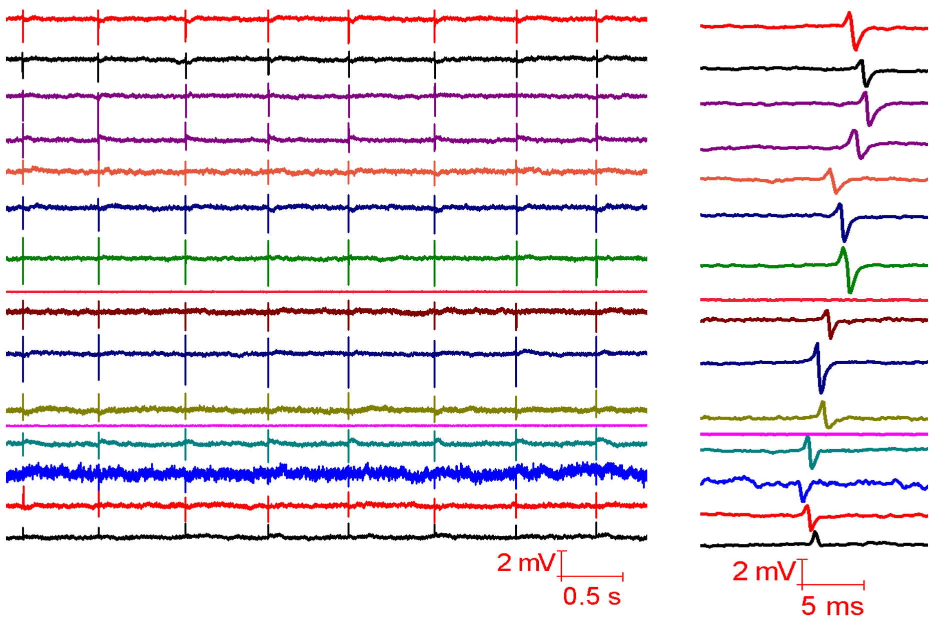
Signal-to-noise ratio is improved compared to ISFETs

J. F. Eschermann et al., Applied Physics Letters, 95, 083703 (2009)

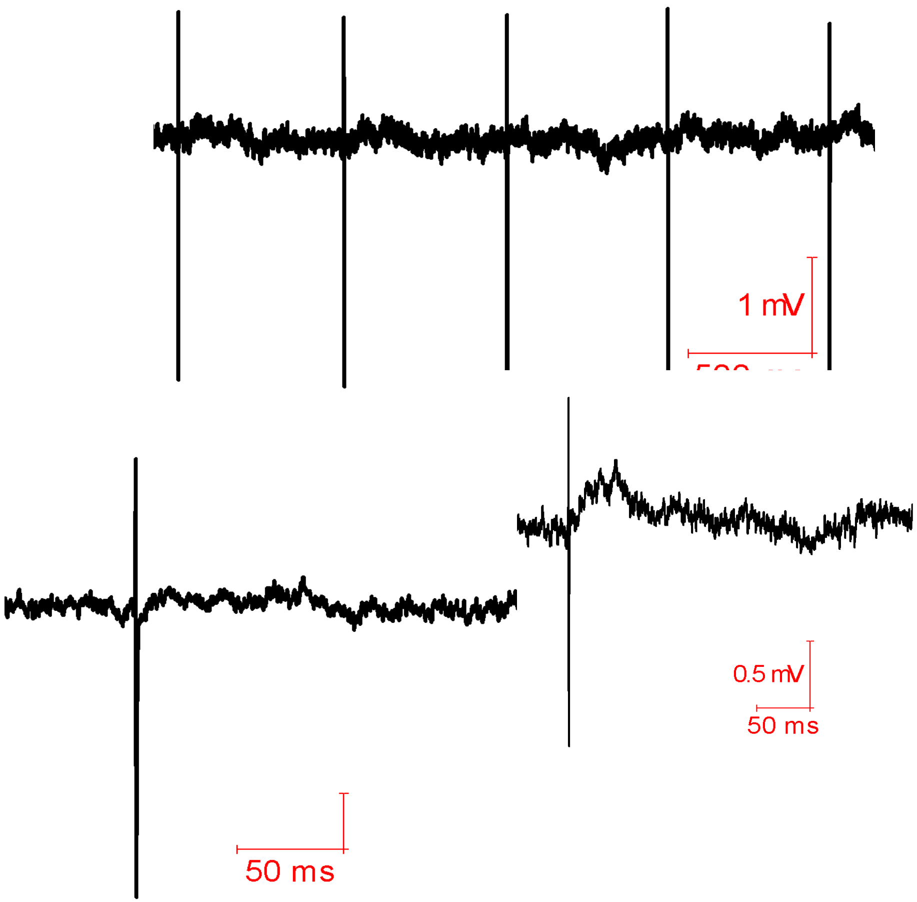
Assay 4: Recording of extracellular action potentials

Results unpublished

Primary cardiac myocyte culture!!!



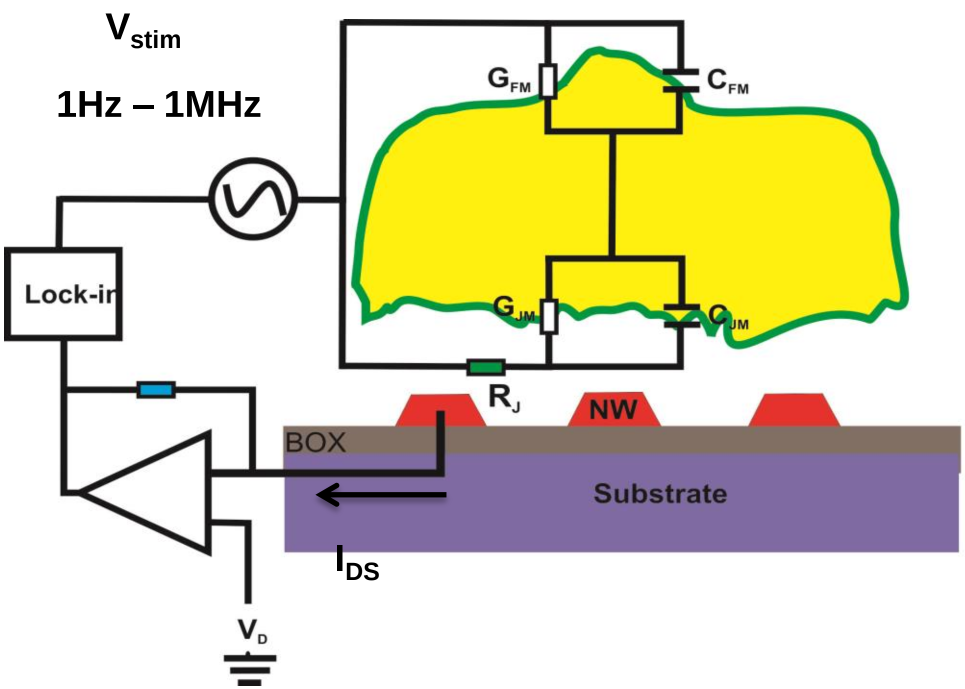
Each of the 16-channels is responding

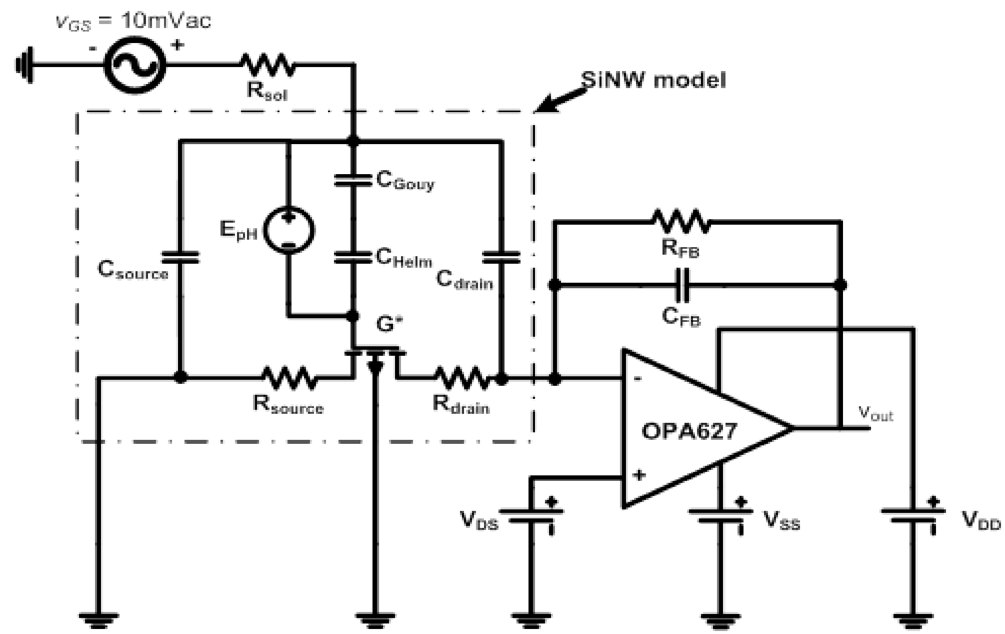


High signal-to-noise ratio : 5 – 10 due to the strong coupling between the cells and the SiNWs

Bioassays
Assay 5: Impedimetric detection of cellular adhesion

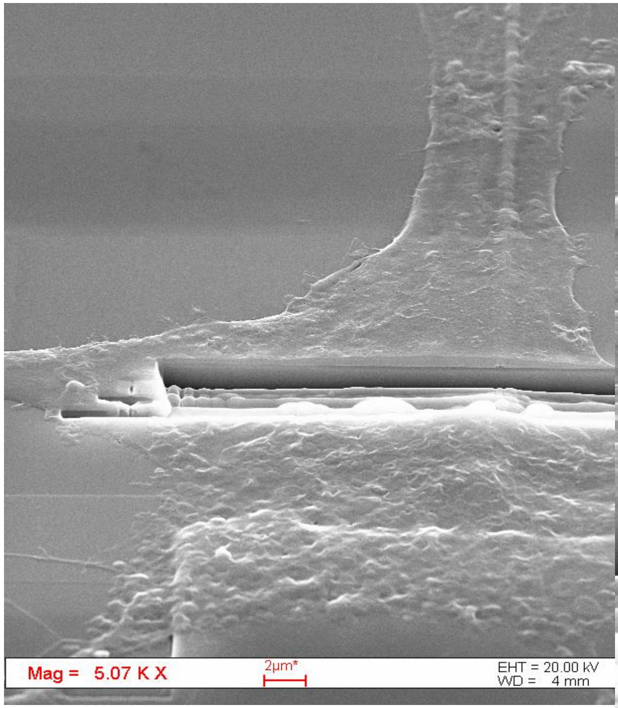
Assay 5: Impedimetric detection of cellular adhesion

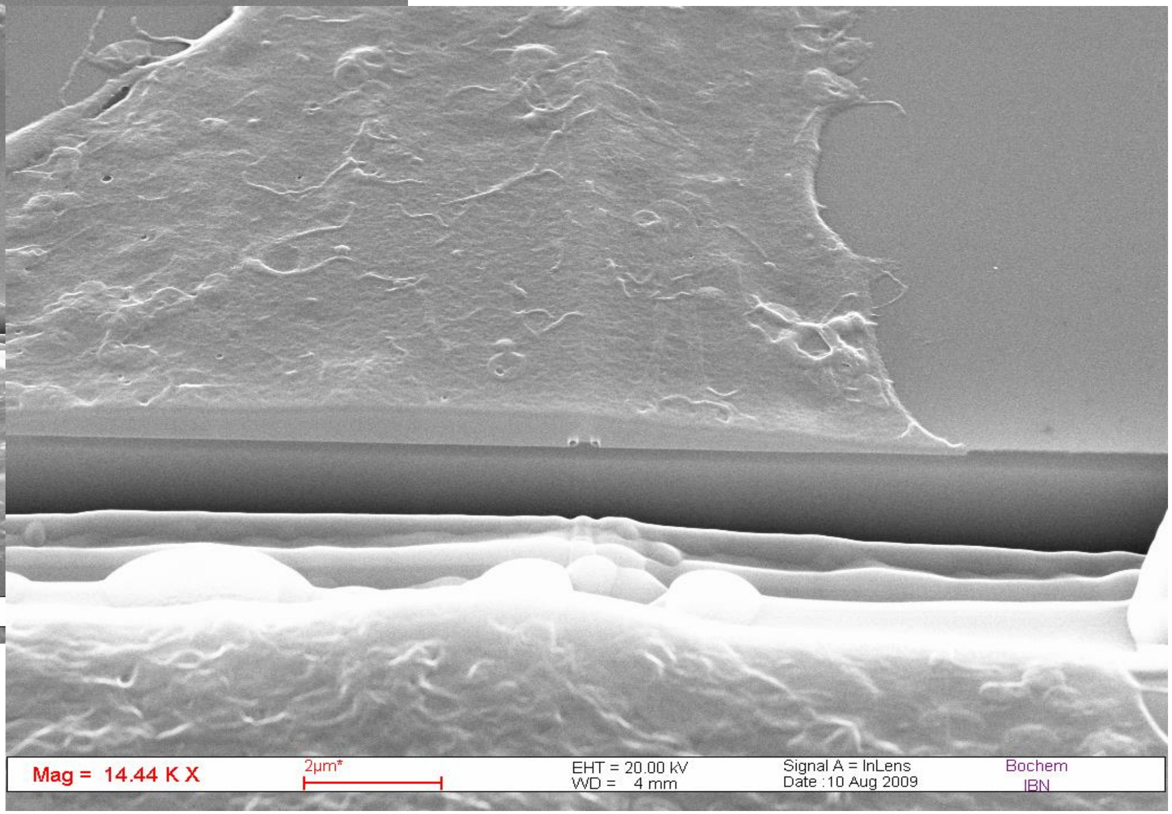




Assay 5: Impedimetric detection of cellular adhesion

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**

Participants

2 Ph.D. students (3 years)

1 (Coordinator)	UBath	N	N	United Kingdom
2	CardiffU	N	N	United Kingdom
3	SAS	N	N	Slovakia
4	INESC-MN	N	N	Portugal
5	DCU	N	N	Ireland
6	AET	Y	Y	United Kingdom
7	EPFL	N	N	Switzerland
8	FH-KL	N	N	Germany
9	Xeptagen	Y	Y	Italy

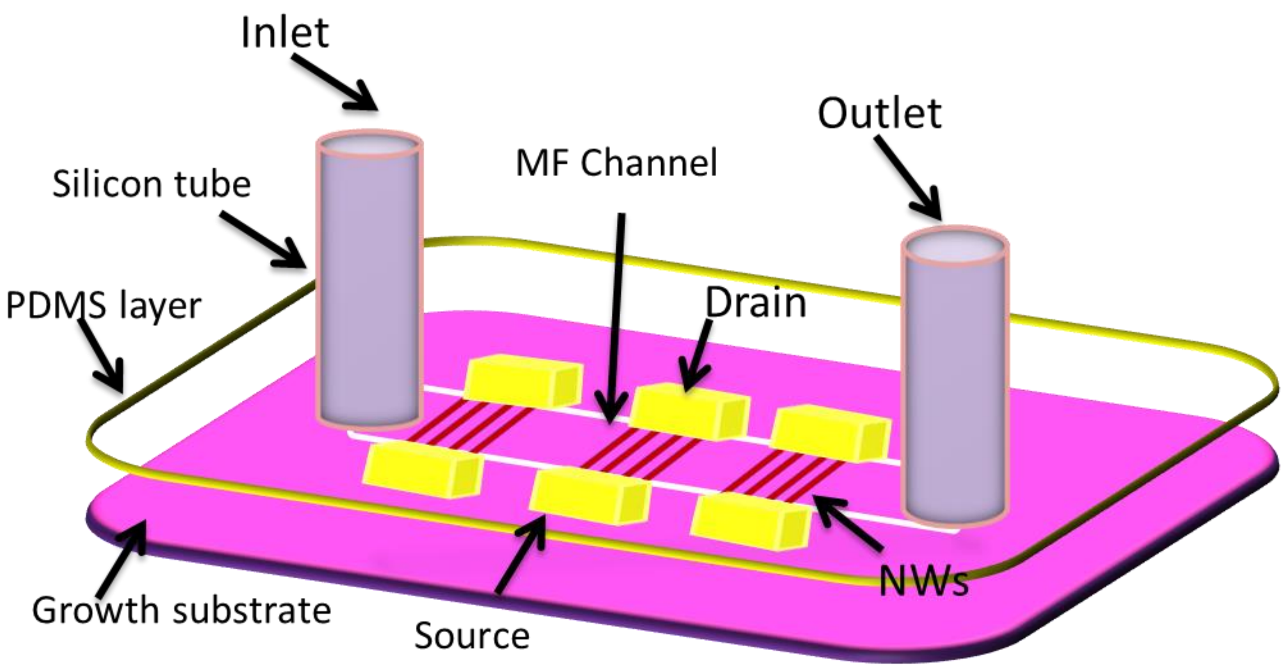
Subject 1: SiNW Sensors
Subject 2: rGO-Sensors

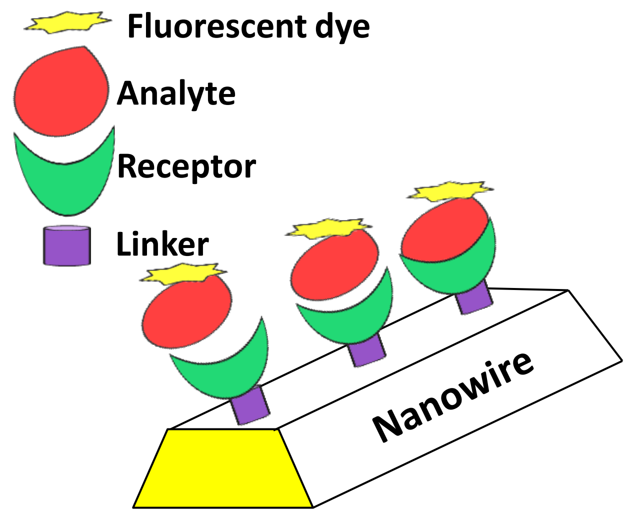
Associated Partners

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

New research projects related to SiNW sensing

Developing a NW-based sensor platform for detection of PCa in parallel with fluorescence techniques



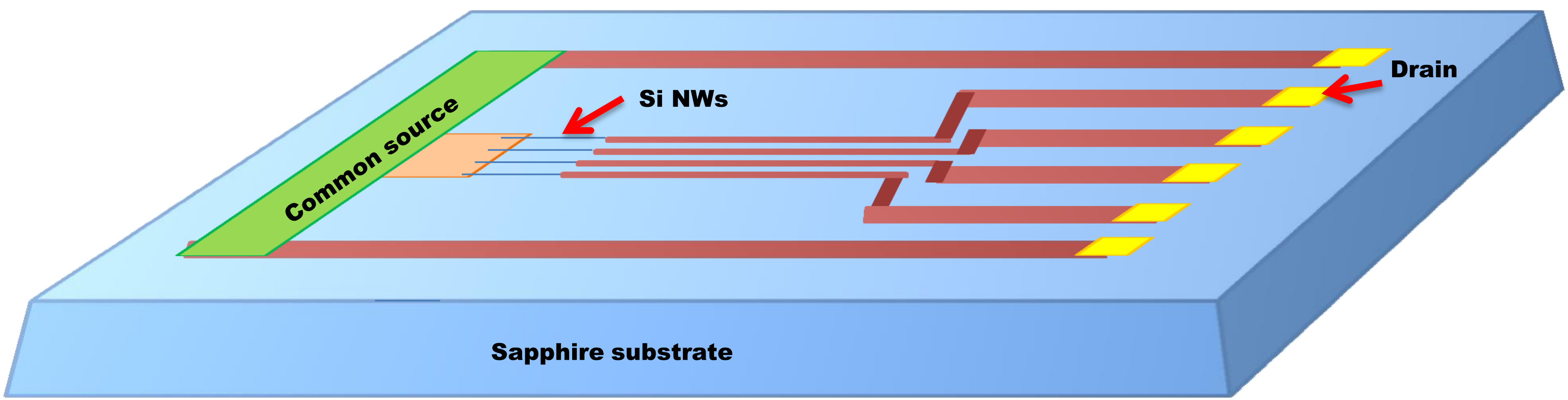


D. Rani, Dr. X.-T. Vu

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*7mm^2 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)

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European Commission

EUROIMMUN Medizinische Labordiagnostika AG

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

DAAD:
PPP exchange grant – Germany/Hong Kong

EU-ITN Marie Curie: Prosense

Industry: ‘Alternative Biosensor Principles’

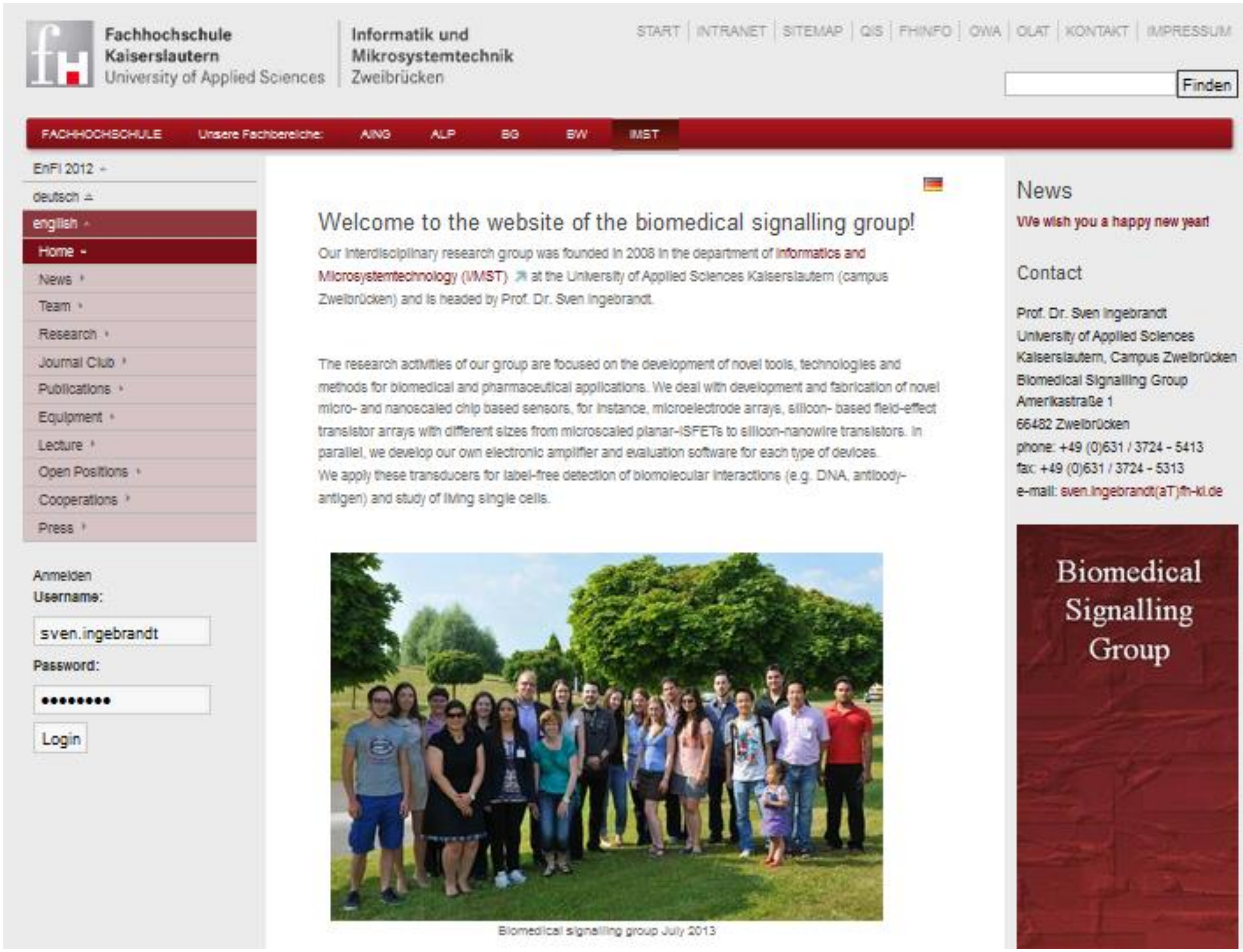
Internal fh-funding:
- Nanotox
- Nanoimprint-Lithography

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

University of Applied Sciences Kaiserslautern



http://www.fh-kl.de/fachbereiche/imst/arbeitsgruppe-bioelektronik/en/home.html