

Prof. Thomas Otto

LAB-ON-CHIP-SYSTEMS

PLATFORMS FOR BIOSENSOR INTEGRATION

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bi.FLOW
systems GmbH
biofluidic integration


TECHNISCHE UNIVERSITÄT
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ZfM
Zentrum für
Mikrotechnologien

 **Fraunhofer**
ENAS

Contents

1. Introduction

2. Lab-on-a-Chip – the Fraunhofer ivD Platform

3. Towards Commercialization

4. Summary

Current Diagnostic Situation

Sample-taking



Sample

Result

Central Lab



Duration of hours to days

Fast diagnosis /analysis at the **“Point-of-Need”** necessary:

- **Emergency** Medicine (Time!)
- Rural Areas, Developing Countries, Territorial States...(Infrastructure!)
- **Catastrophy**, Pandemia (Infrastructure!)
- Tests of Patients for Compatibility/Efficiency of drugs before treatment (→ **“Personalized Medicine”**)
- **Environmental / Food** analyses

Quicktests – Lateral Flow Stripes



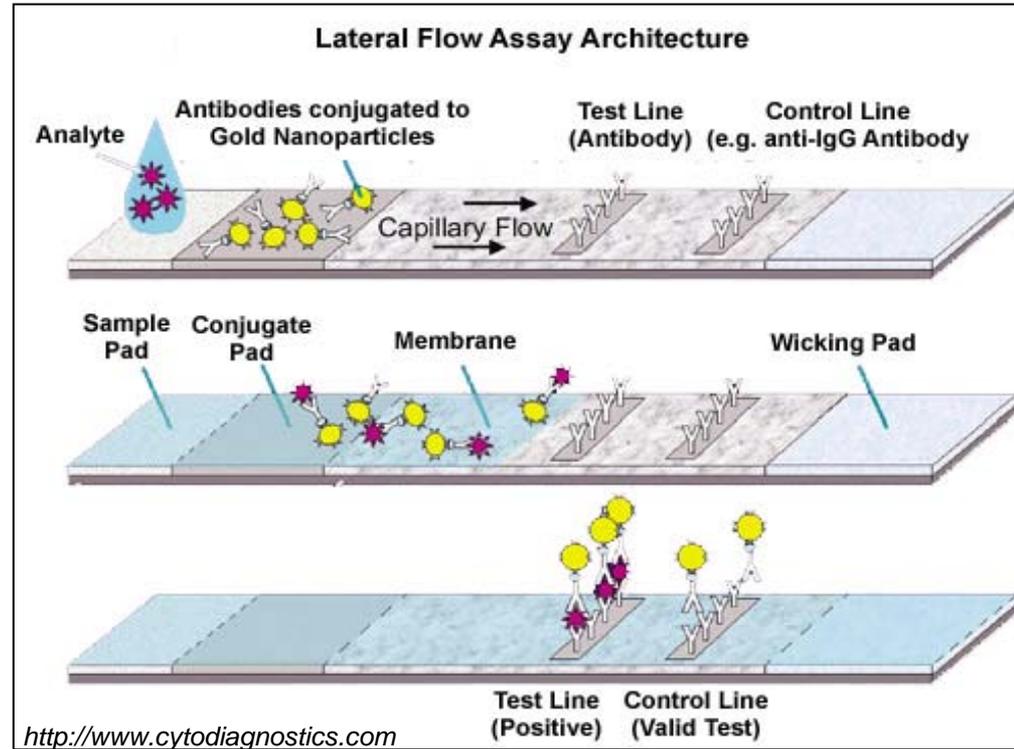
www.onthespotdrugtest.com



sww-entwicklung.de



www.merck-chemicals.de



- low-cost
- mainly for qualitative/semi-quantitative analyses
- only „simple“ assays
- some of them need manual sample prep in advance

Quicktests - with even more fluidics

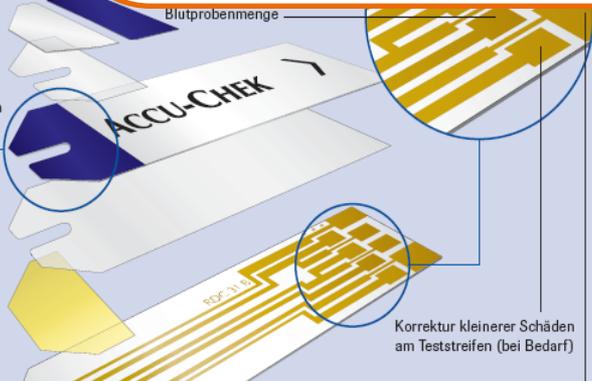
- Quicktests typically do not integrate sample preparation
- Current quicktests make no use of advanced biosensor capabilities (i.e. for multi-parameter analyses)
- Advanced Biosensors require more complex (fluidic) protocols

Test stripe

Der für einen einfachen Blutprobenauftrag konzipierte Teststreifenrand saugt das Blut schnell und problemlos ein.

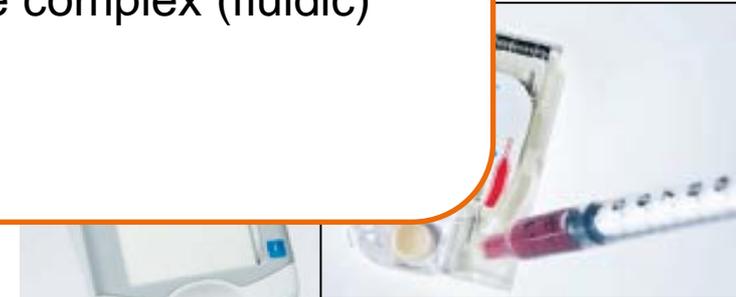
Der starke Blau-Gelb-Kontrast bietet einen klaren Anhaltspunkt beim Auftragen der Blutprobe.

Die breite Y-förmige Auftragszone sorgt für ein punktgenaues Auftragen und ein schnelles Einziehen der Blutprobe.



Transport by **capillary forces**
(for simple protocols)

Blood glucose measurement
(Roche AccuCheck)



Transport by **external punching**
(protocols with few/only one steps)

Multiple Blood parameters
(Abbot PoC - iStat)

Contents

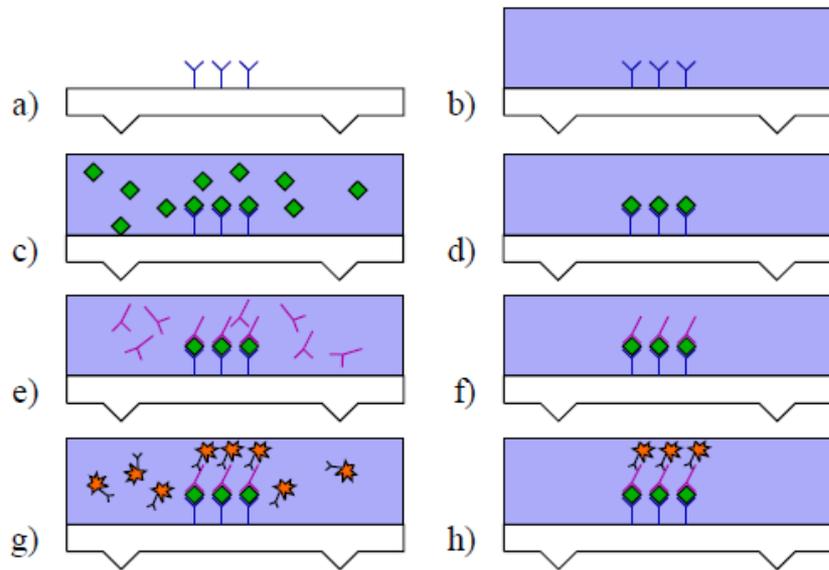
1. Introduction
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A Biotech-Protocol

Immuno-Assay with Biosensor

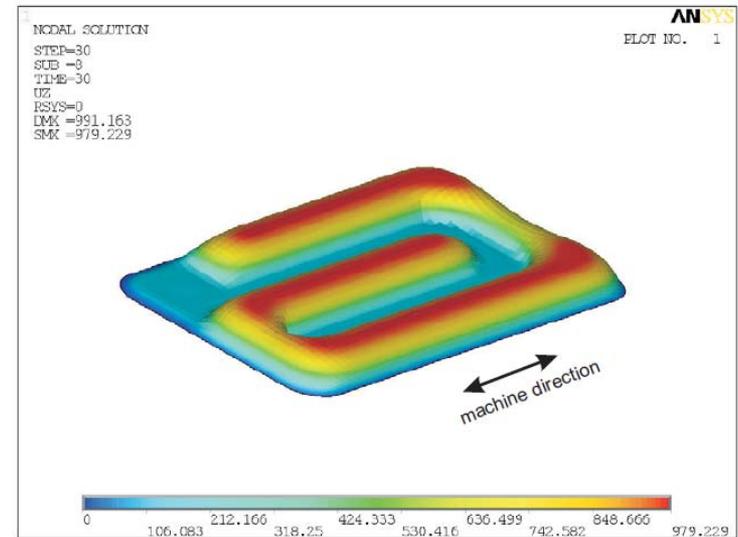
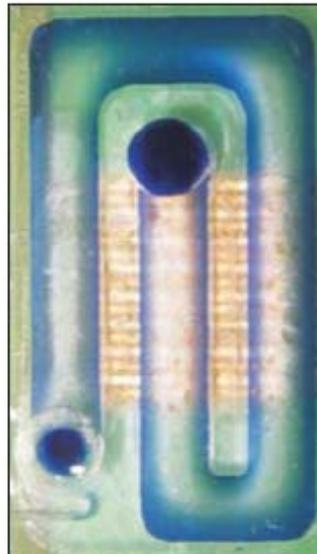
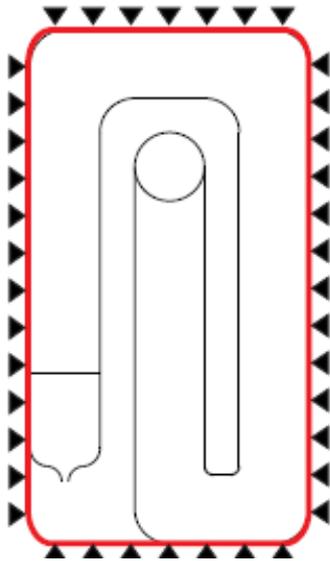
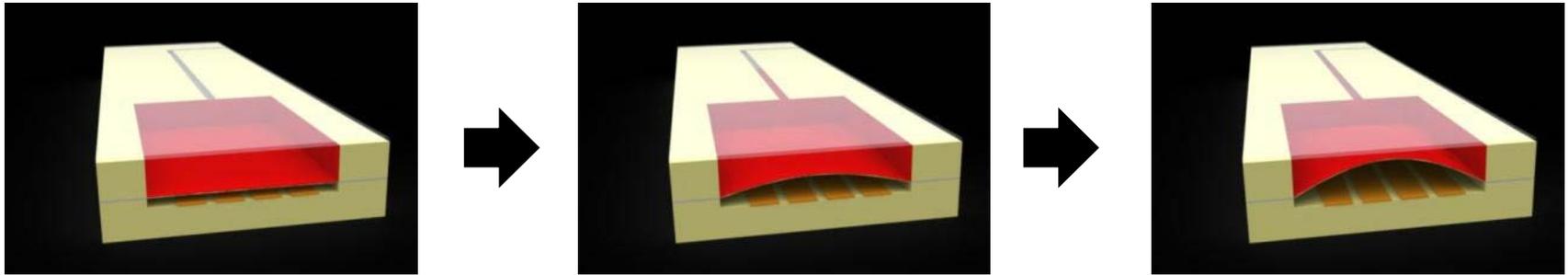
- a) Initial State
- b) Buffer (Washing step)
- c) Sample with Antigen
- d) Buffer (Washing step)
- e) Monoclonaler Antikörper
- f) Buffer (Washing step)
- g) Fluoreszenz-markierter Antikörper
- h) Buffer (Washing step)

Liquid handling / pumping



Integrated Pumping

Principle: A **membrane** gets **deformed** by an electrolytically **generated gas pressure**. The membrane deformation leads to a displacement of the liquid out of the reservoir above the membrane

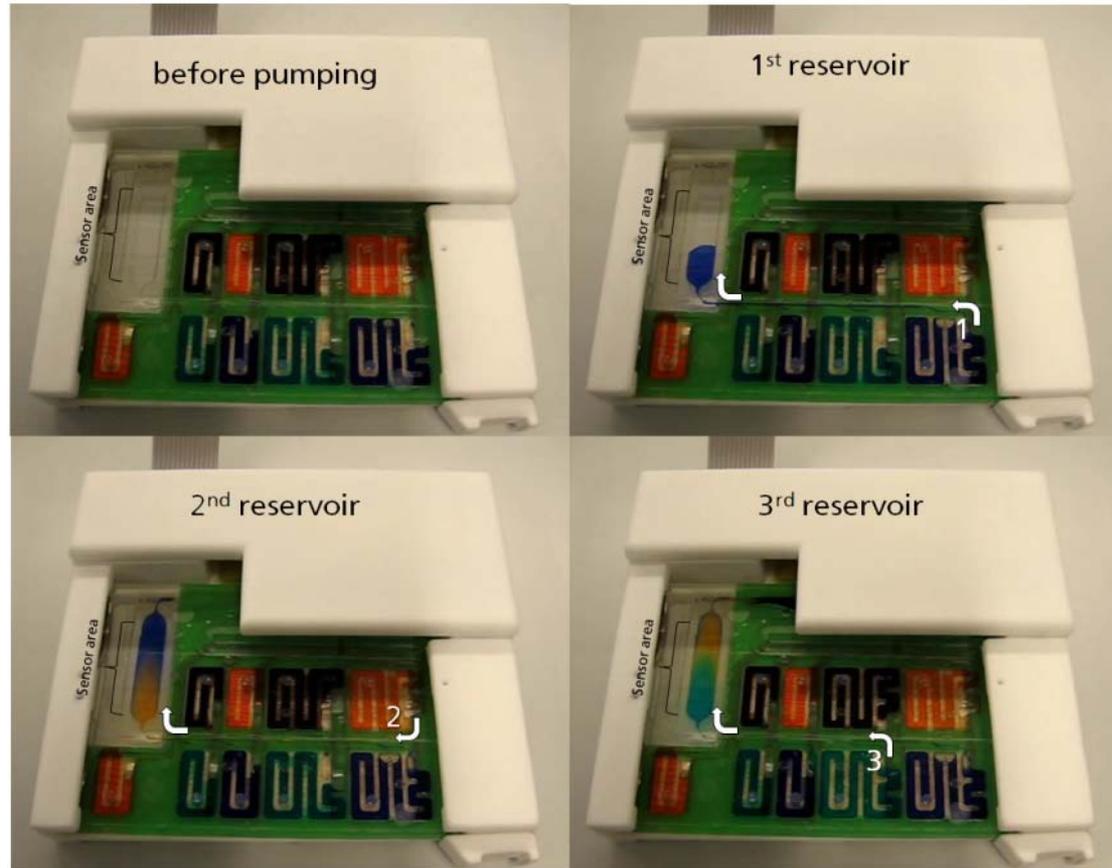


Functions: Integrated Pumping

Bioassays can be run **completely automatically** by integrated low-cost pumping functionality

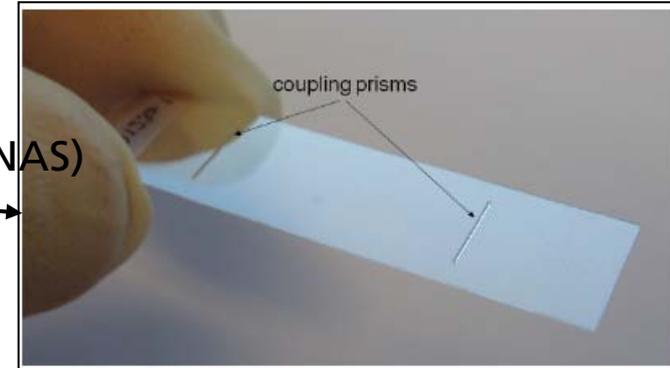
→ **Picture shows the emptying of the first 3 reservoirs** by the integrated micropumps

→ Cartridge has **only electrical interface** to the outside world

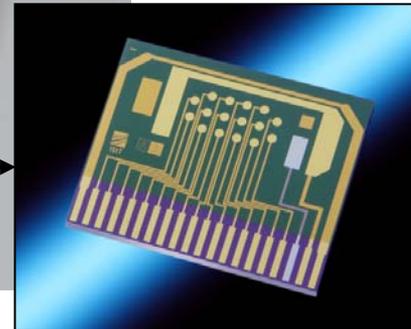


Integration of multiple sensor principles

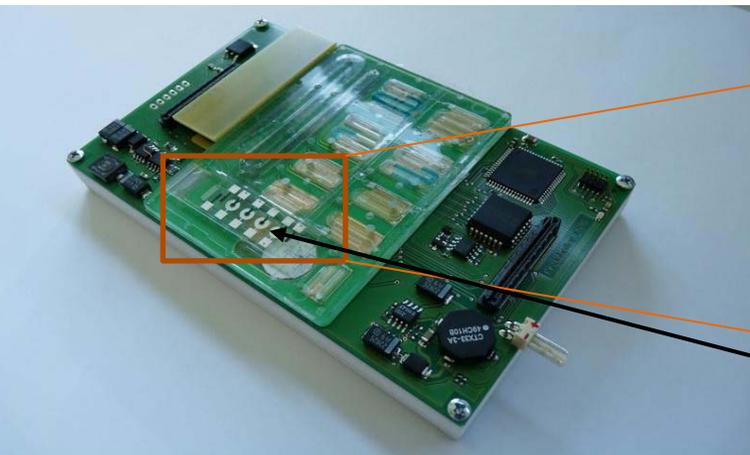
**Polymer-based
Optical TIRF sensor (Fh IPM, ENAS)**



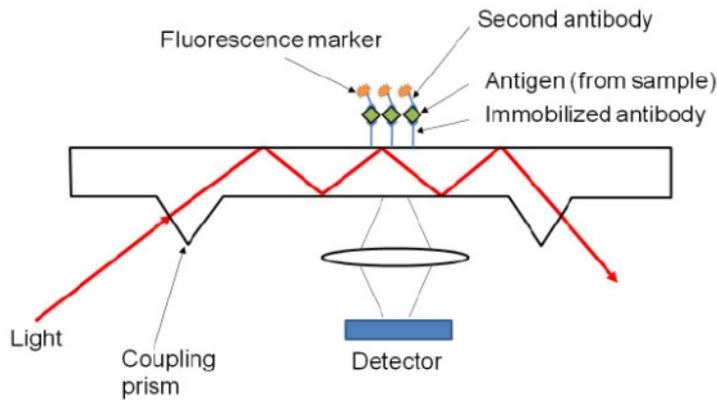
**Si-based
electrochemical sensor (Fh ISIT)**



**amperometric/voltametric/conductivity sensor
screen-printed on polymer foil**

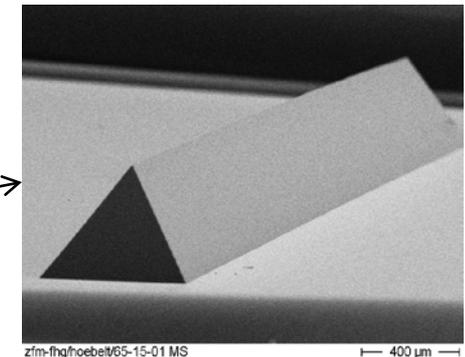
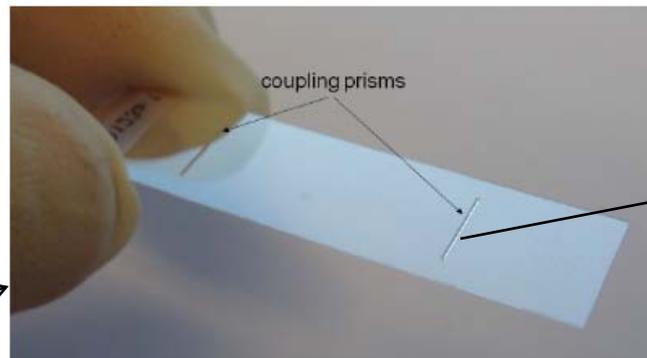
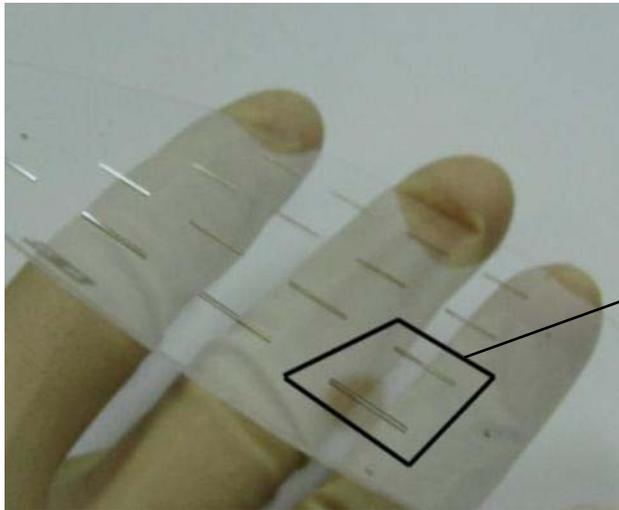


Sensor 1: Optical biosensor - TIRF



TIRF= total internal reflection
fluorescence

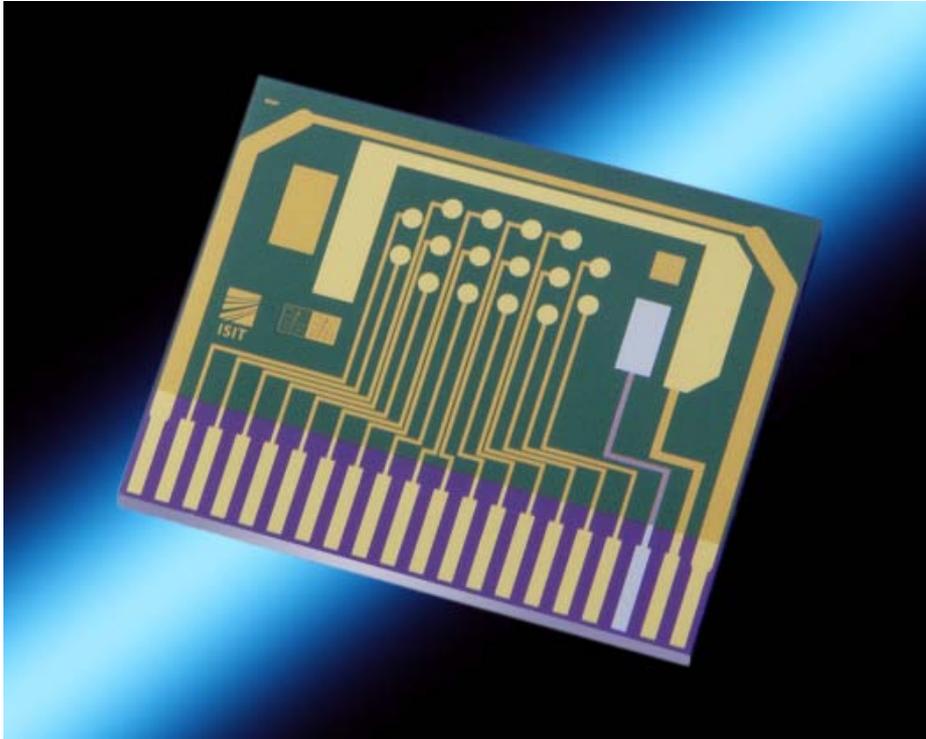
- Fully polymer based
- Fabricated by injection moulding or hot embossing (as batch process)
- Low-cost, highly sensitive sensing principle



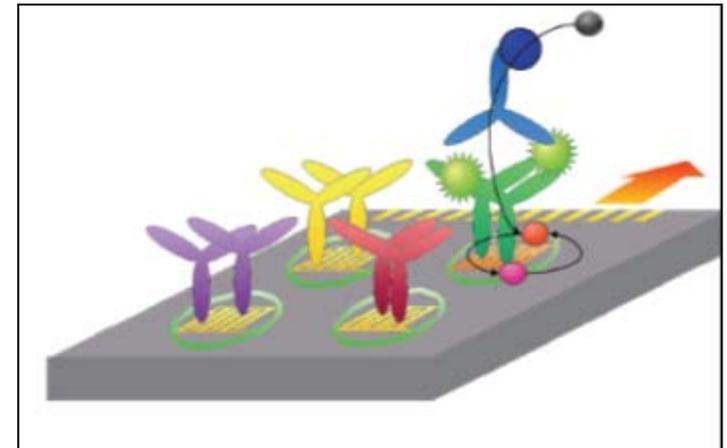
Hot-embossed TIRF sensor
(130μm thick polymer foil with 200..300μm high coupling prisms)

For further details on sensor & readout instrument see
Brandenburg, Curdt, Nestler, Otto, Wunderlich, Michel, Proc. SPIE Photonics West, 2009

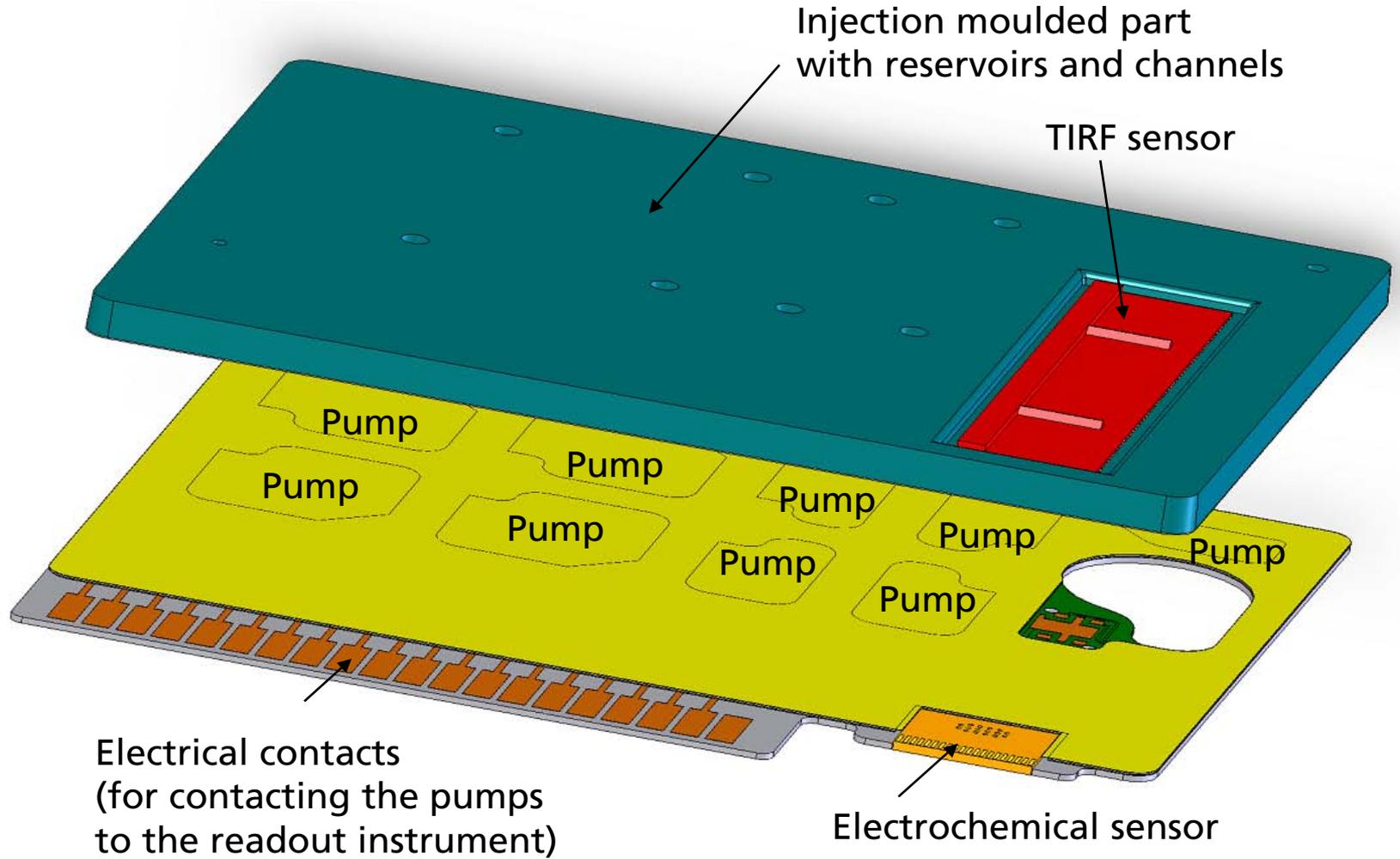
Sensor 2: Electrochemical sensor



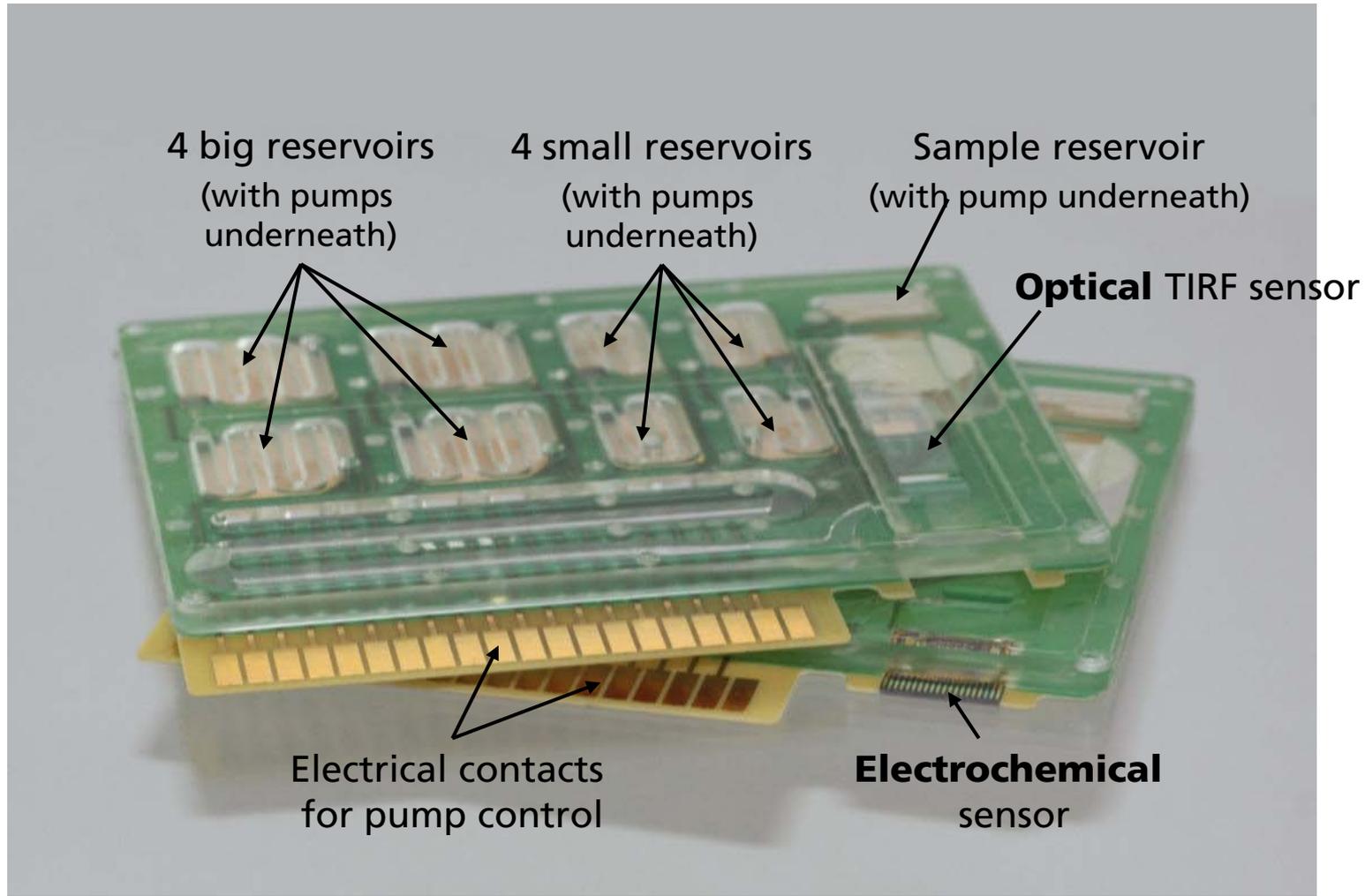
Electrochemical Biosensor
based on Redox Cycling
→ Silicon technology (ISIT)



Cartridge layout (with pumps and sensors)



Complete cartridges (with pumps and sensors)



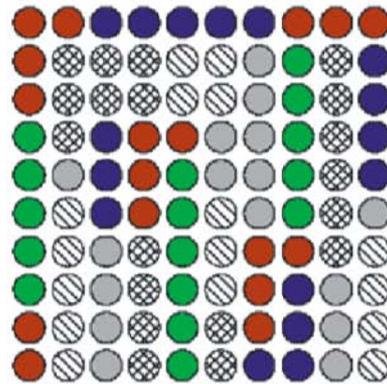
Sample BioAssay: CRP- and PSA Immunoassay (ELISA)

Readout Instrument (Fraunhofer IPM) and CRP assay (Fraunhofer IBMT)



Sequence:

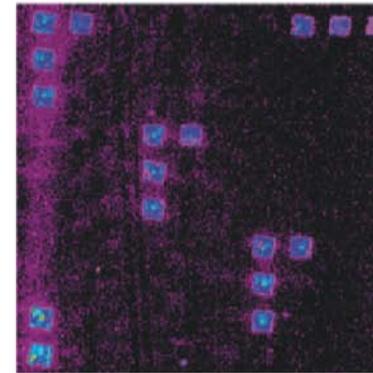
1. Washing
2. Sample
3. Washing
4. Antibody 1
5. Washing
6. Antibody 2 (labeled)
7. Washing



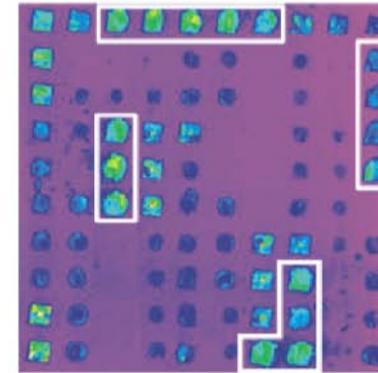
Legend

- Guide spots
- Negative control
- PSA
- CRP
- Hormon 1
- Hormon 2

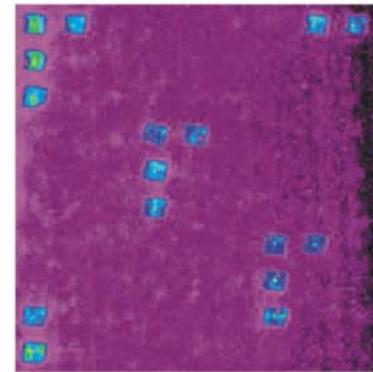
a) Spotting layout



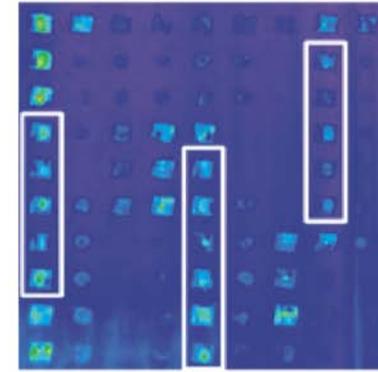
b) CRP before assay



c) CRP after assay



d) PSA before assay



e) PSA after assay



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BiFlow Systems in a nutshell: Competencies



- Microfluidics
- Systems Integration

- Diagnostics
- Biomedicine



BiFlow Systems in a nutshell: BioFluidic Integration

Flexible Microfluidic Platforms not requiring any tubing or external pumps

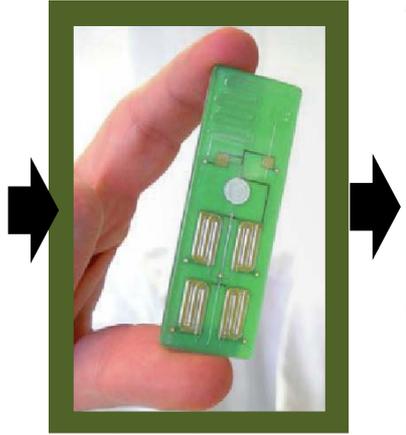
- ✓ Integrated reservoirs and micropumps
- ✓ Low-Cost polymer Lab-on-Chip platforms
- ✓ Standard products ready for evaluation



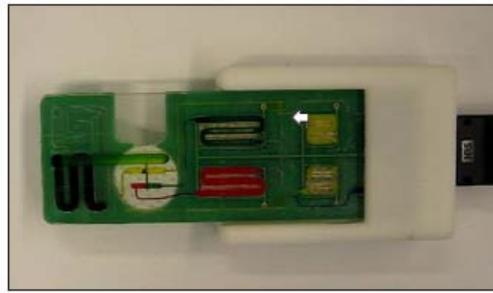
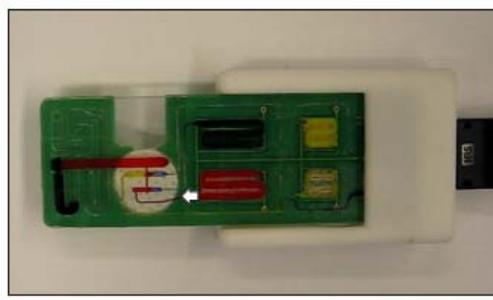
No Tubing



No External Pumps

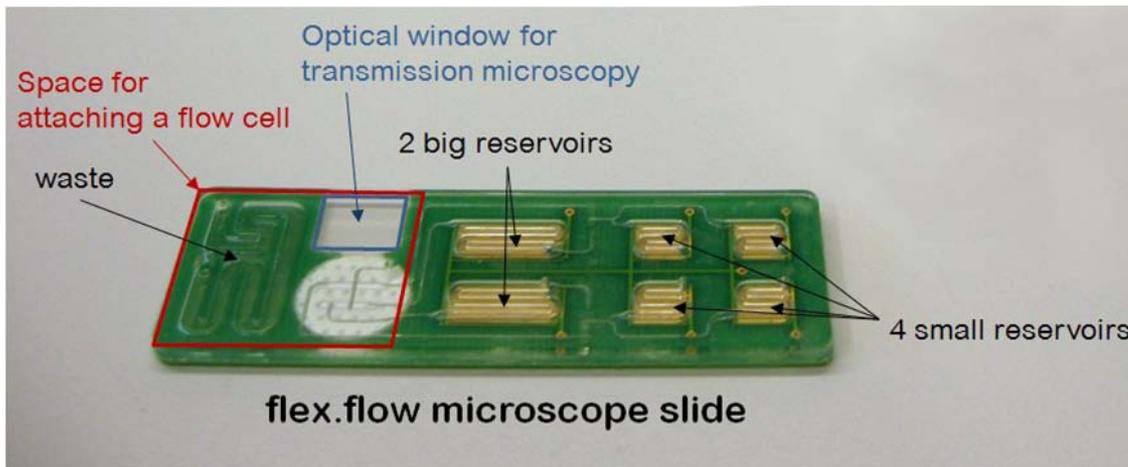


On-Chip
Pumps + Reagents



flex.flow: Self-pumping Microscope Slides

- **Active microscope-slides** for R&D
- Allows **technology evaluation** for assay miniaturization and biosensor integration



Configurations:

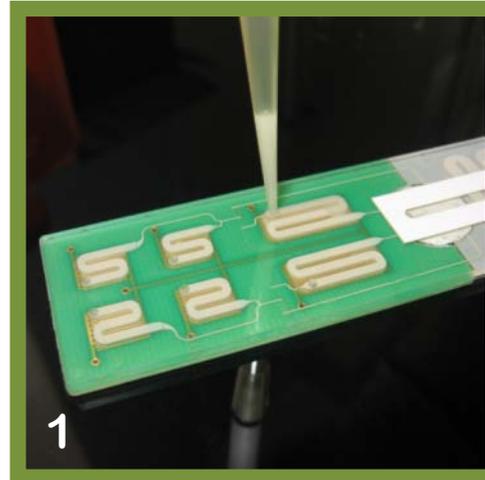
- 4+2 reservoirs
- 4+1 reservoirs + 1 mixing reservoir
- 4 reservoirs + 2 mixing reservoirs
- **Flow-Cells** for attaching different **biosensors**
- **Flow-Cells** for attaching/detaching a **Microscope Slide**

flex.flow: Running a CRP assay

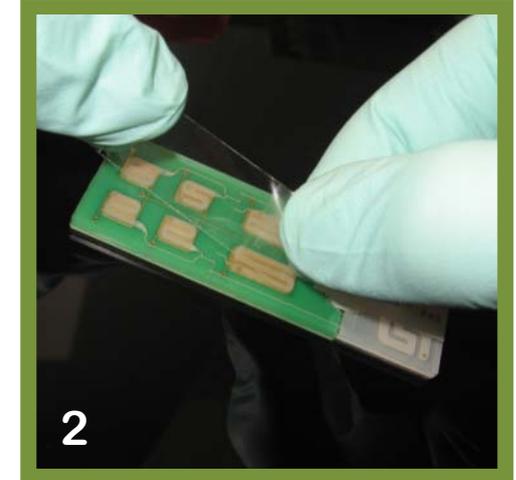
Preparation



Start
flex.flow microscope slide
with flow-cell tape



Filling the reservoirs
with reagents, washing
solutions and sample
using a standard pipette tip

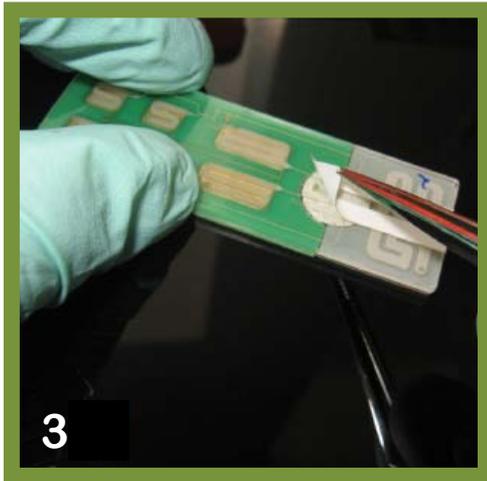


Sealing of the filling holes
with a tape

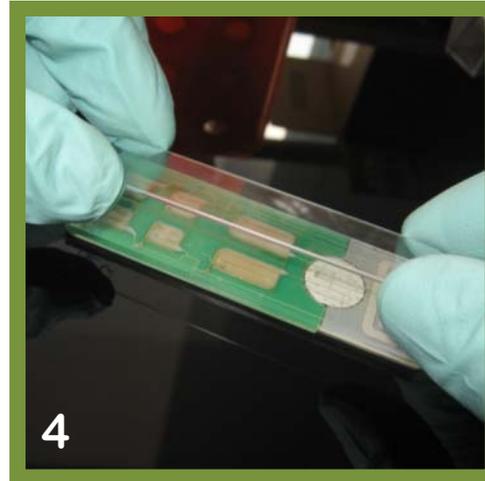
Reservoir 1: CRP sample
Reservoir 2: antiMouse (Cy5-labeled)
Reservoir 3: Buffer
Reservoir 4: Deionized water
Reservoir 5: Buffer
Reservoir 6: antiCRP (monoclonal, mouse)

flex.flow: Running a CRP assay

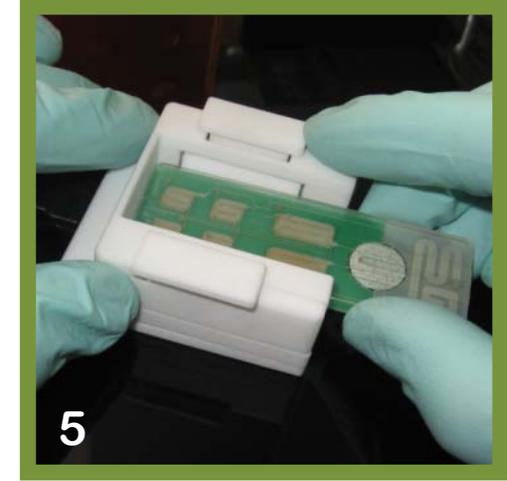
Preparation



Detachment of protection layer of flow-cell tape.



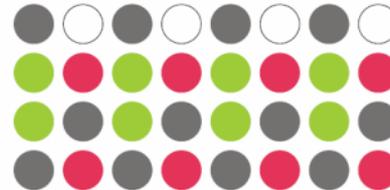
Microarray slide attachment



Insertion into Adapter

Spotting pattern of microarray slide

- antiCRP (polyclonal, goat) 1 mg/mL
- antiHCG-a (polyclonal, goat) 0.5 mg/mL
- antiMouse (Cy5-labeled, sheep) 5 µg/mL
- carbonate buffer (200 mM, pH 9.6)



flex.flow: Running a CRP assay

Programm and run the Assay

Zeitsteuerung - bi.FLOW Pumpensteuerung

Name | max. | Status | validiert

Interval [s]	0	1	2	3	4	5	6
Pumpe1	20,0 µl	0,20 µl	0,2 µl				
Pumpe2	20,0 µl	20,00 µl	0,0 µl			1	
Pumpe3	40,0 µl	23,20 µl	5,8 µl		0,87		
Pumpe4	40,0 µl	40,00 µl	11,2 µl				0,96
Pumpe5	20,0 µl	16,70 µl	0,2 µl		0,66		
Pumpe6	20,0 µl	20,00 µl	0,2 µl			0,66	

Time step number
Enter duration of each time step

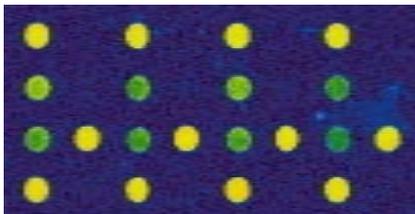
Enter flow rates for each pump and time step

Name	max.	Status	validiert	0	1	2	3	4	5	6
Interval [s]				10	30	30	30	20	20	30
Pumpe1	20,0 µl	0,20 µl	0,2 µl		0,66					0
Pumpe2	20,0 µl	20,00 µl	0,0 µl		0				1	
Pumpe3	40,0 µl	23,20 µl	5,8 µl	1,68	0			0,87		
Pumpe4	40,0 µl	40,00 µl	11,2 µl							0,96
Pumpe5	20,0 µl	16,70 µl	0,2 µl			0,66				
Pumpe6	20,0 µl	20,00 µl	0,2 µl				0,66			

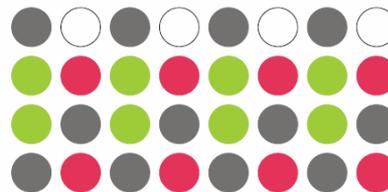


- 0) Res. 3 Buffer
- 1) Res. 1 CRP sample
- 2) Res. 5 Buffer
- 3) Res. 6 antiCRP (monoclonal, mouse)
- 4) Res. 3 Buffer
- 5) Res. 2 antiMouse (Cy5-labeled)
- 6) Res. 4 Deionized water

Getting your result



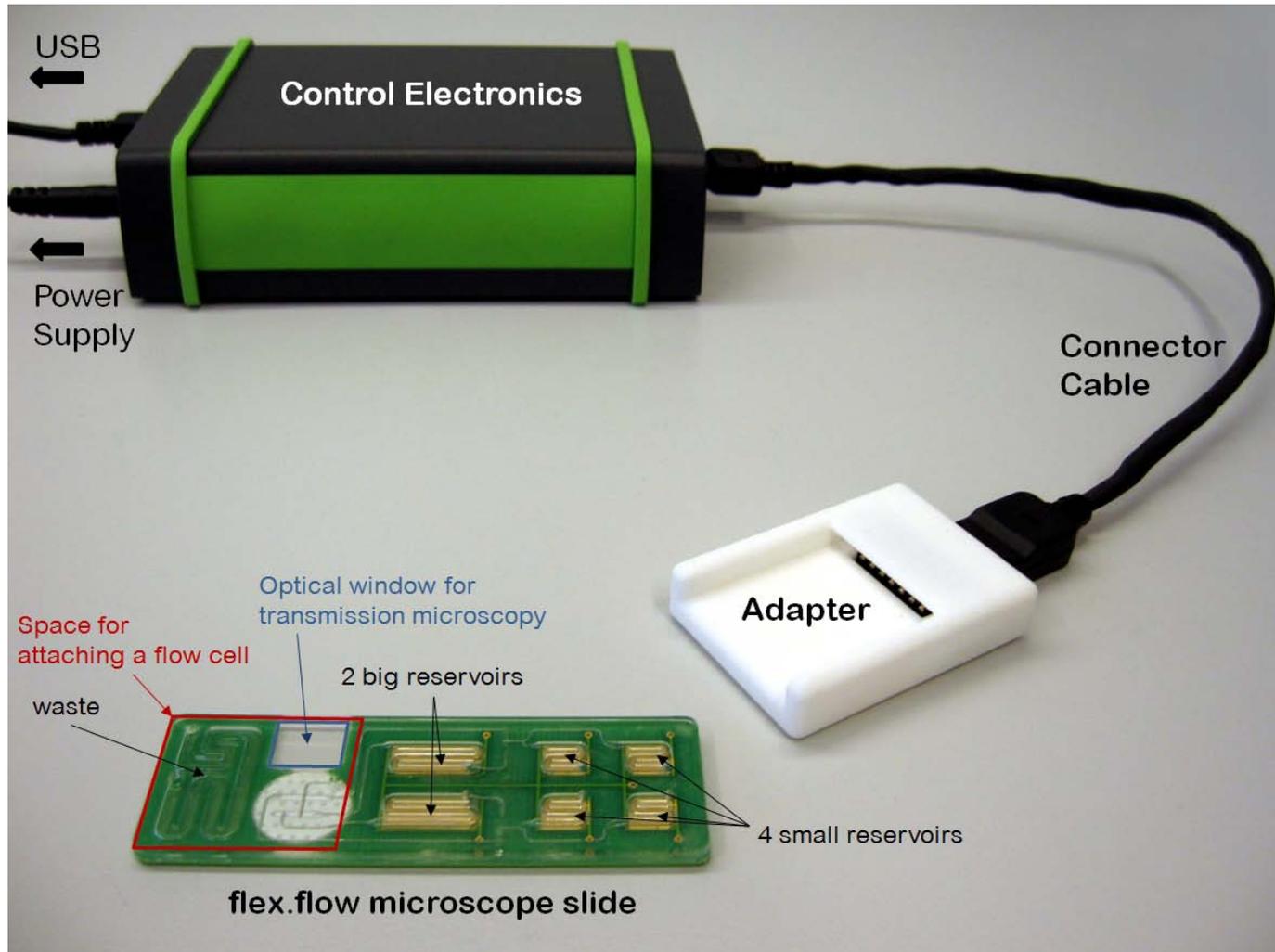
Readout by conventional microarray reader



Spotting pattern

- antiCRP (polyclonal, goat) 1 mg/mL
- antiHCG-a (polyclonal, goat) 0.5 mg/mL
- antiMouse (Cy5-labeled, sheep) 5 µg/mL
- carbonate buffer (200 mM, pH 9.6)

flex.flow: Evaluation Kit

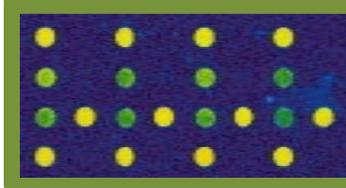
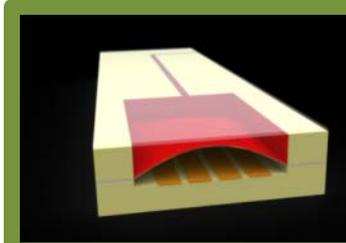
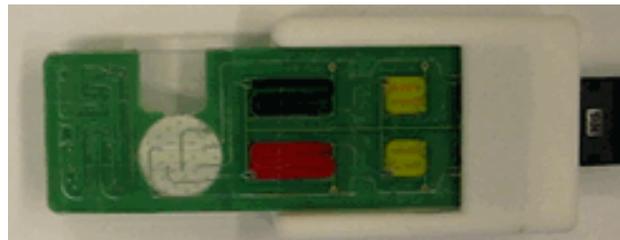


Includes:

- Control Electronics
- Adapter
- Software
- 10 flex.flow Microscope Slides

bi.flow systems

- **Standard Products:**
 - **Microscope slides** with integrated reservoirs and micropumps for **biosensor integration** and **assay miniaturization**
- **Possible Partnerships:**
 - **Biosensor** and **Bioassay developers** and manufacturers looking for smart ways of integrating their biosensors / their bioassays in a self-contained microfluidic chip (contractual development or research projects)



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Summary

- Lab-on-chip platform for assay miniaturization
- Open for the integration of different biosensor principles
- Commercialized solutions available via BiFlow Systems GmbH

THANK YOU VERY MUCH
VIELEN DANK