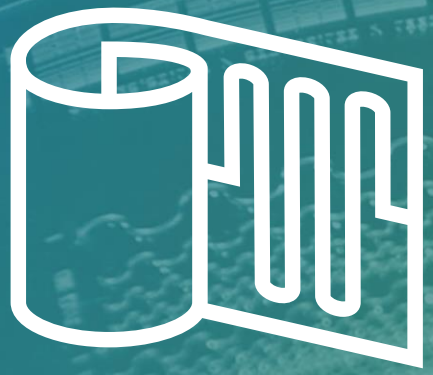


Microfluidics InnovationHub

We get Microfluidics rolling

NextGenMicrofluidics (NGM)

- NGM is an Open Innovation Test Bed
- 21 companies
- worldwide biggest platform for upscaling and testing of microfluidic devices

Design & Simulation

BIONIC SURFACE TECHNOLOGIES

Materials

condensia

micro resist technology

Electronics manufacturer

infineon

Bioprocess

NST

Process development

scienion

INMOLD

temicon

RESCOLL

Microfluidics development & manufacturing

bi.FLOW systems

micronit microtechnologies

Medical senors

Erba Technologies Austria GmbH

Erba Mannheim

GENSPEED BIOTECH

Cell culture solutions

ibidi cells in focus

Innoprot Speeding up drug discovery

Research

tecnalia Inspiring Business

TU Graz

BRFAA

BNN

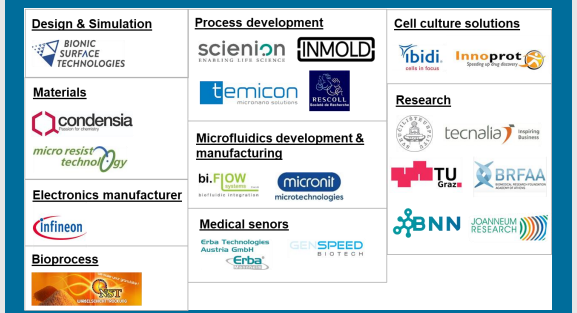
JOANNEUM RESEARCH

Microfluidics InnovationHub

MIH – Single Entry Point to NGM Services

MIH is a non-profit association that includes 20 NGM members

Research
Development
Production



Program Management

Partner Coordination

Single Entry Point

MIH Operations

Marketing & Sales

Business Fulfillment

MARKETS

USE CASES

CUSTOMERS

EXTERNAL PARTNERS

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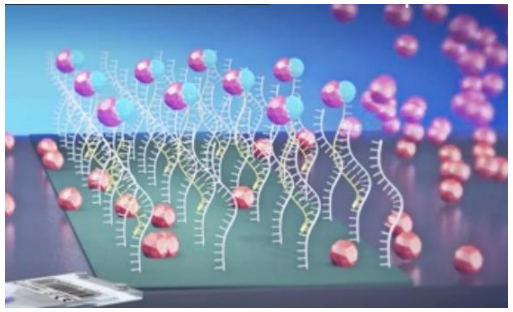
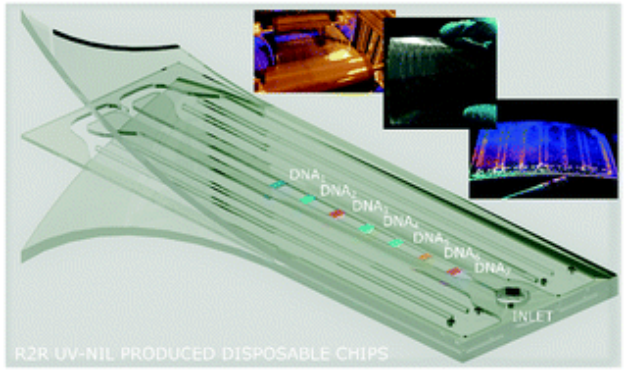
MIH Service Portfolio

Primary Components

Assay | Design | Patterning | Surface functionalisation | Electrodes Sensors | Backend Services | Read Out Device System Integration | QM



IVD - ELISA, LAMP, PCR
ENZYME Detection
CELL analysis
Water, Food analysis



Active & Passive Microfluidics
Single units to millions of units
From Milling over Inj. Mold to Roll-to-roll

Microfluidic Chip

Microfluidic Solution

Microfluidics InnovationHub

Microfluidics – Value Chain

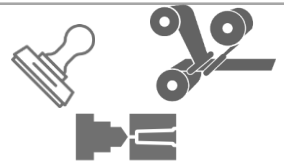
Microfluidic Chip

Microfluidic Solution

Primary Components

Assay → Design → Imprinting → Surface functionalisation → Electrodes Sensors → Backend Services → Read Out Device System Integration → QM







Industrial

micro resist technology

BIONIC SURFACE TECHNOLOGIES

RESCOLL Société de Recherche

micronit microtechnologies

temicon micronano solutions

micronit microtechnologies

scienion ENABLING LIFE SCIENCE

temicon micronano solutions

INMOLD

micronit microtechnologies

bi.FLOW systems biofluidic integration

bi.FLOW systems biofluidic integration

bi.FLOW systems biofluidic integration

micronit microtechnologies

infineon

micronit microtechnologies

GENSPEED BIOTECH

bi.FLOW systems biofluidic integration

Erba Technologies Austria GmbH

Erba Mannheim

NST

RTOs

BRFAA

JOANNEUM RESEARCH

JOANNEUM RESEARCH

JOANNEUM RESEARCH

JOANNEUM RESEARCH

JOANNEUM RESEARCH

JOANNEUM RESEARCH

tecnalia Inspiring Business

TU Graz

ibidi cells in focus

Innoprot Speeding up drug discovery

tecnalia Inspiring Business

condensia Passion for chemistry

tecnalia Inspiring Business

BIO NANONET

Microfluidics InnovationHub

Microfluidics InnovationHub

WE DEVELOP AND PRODUCE

Microfluidic Lab-on-a-Foil Systems

- Single entry point to research & development services
- Comprehensive service portfolio
- Fast prototyping and scale up
- Multiple funding opportunities
- Quality assurance

We offer funding to scale up your application

- ✓ Open Call applications accepted on a rolling basis until September 2023
- ✓ Access to all services of the EU Horizon Europe project NextGenMicrofluidics
- ✓ Funding rate of up to 92% for European SMEs and 50% for Large Enterprises

Microfluidics Innovation Hub is the single entry point of the European project NextGenMicrofluidcs (www.nextgenmicrofluidics.eu). NextGenMicrofluidics has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.

Microfluidics InnovationHub

Webinar: Lab-on-a-Chip for Molecular Diagnostics

29 SEP 2022, 13:00 CEST

Presented by Jörg Nestler

Founder & Managing Director

BiFlow Systems GmbH

bi.FLOW systems GmbH

biofluidic integration

www.microfluidicshub.eu

Content

- **BiFlow Systems company profile**
- **Lab-on-a-Chip (for molecular diagnostics)**
  - Towards Integration
  - Towards Multiplexing
  - Towards Speed
- **Examples**
  - Bacterial species identification / AMR testing
  - Respiratory diseases

BiFlow Systems GmbH

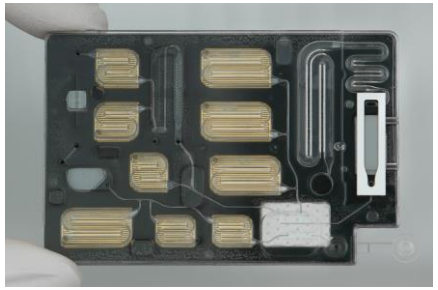
- Founded in 2011
- 18 Employees
- 250m² manufacturing space

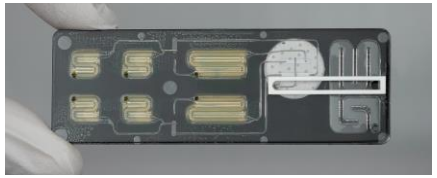
- <u>Contact:</u>
  BiFlow Systems GmbH
  Technologie-Campus 1
  09126 Chemnitz, Germany

  www.biflow-systems.com

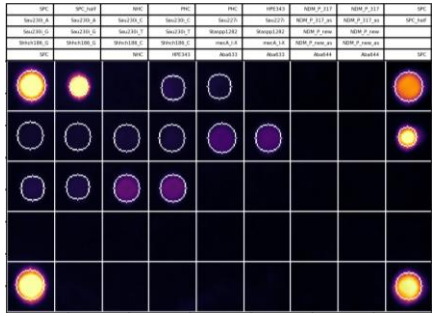
BiFlow Systems GmbH

Microfluidic Cartridges

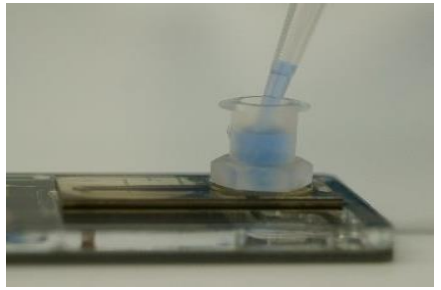


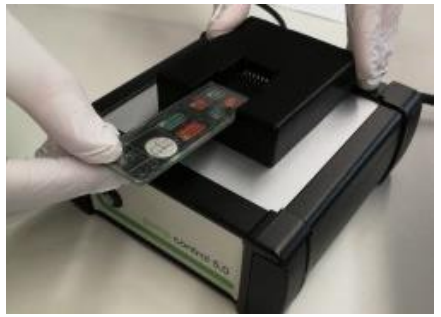


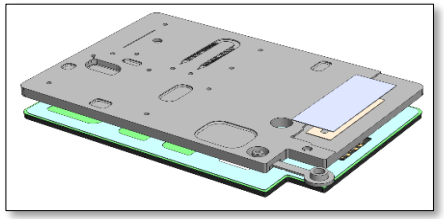
Instrumentation



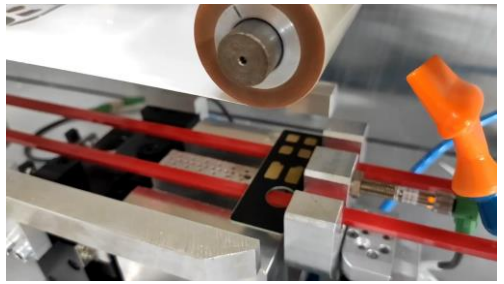
Custom solutions







Fabrication



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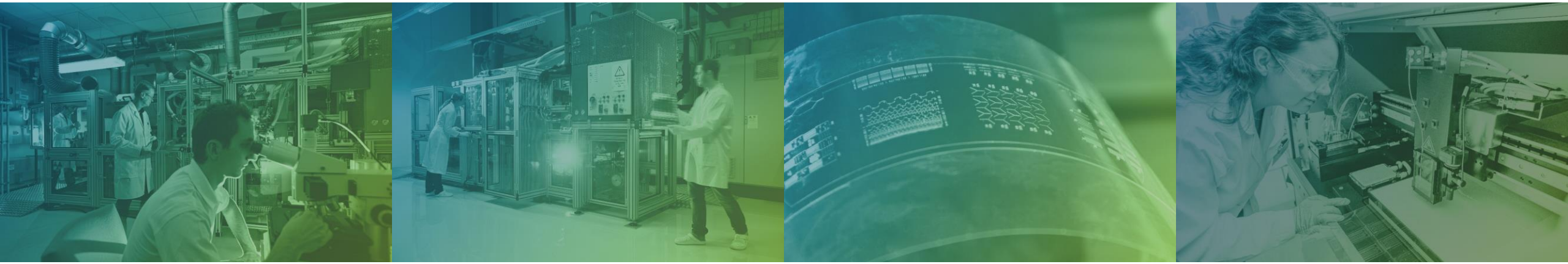
Lab-on-a-Chip
- some introductory words -



Lab-on-a-Chip

- **Lab-on-a-Chip (LoC) =**
  Small microfluidic system that can perform one or more laboratory functions
- **It is typically larger than a „chip“ !**
- **Today, we will**
  - focus on LoC for Molecular Diagnostics (DNA/RNA-based)
  - do NOT talk about lateral flow strips

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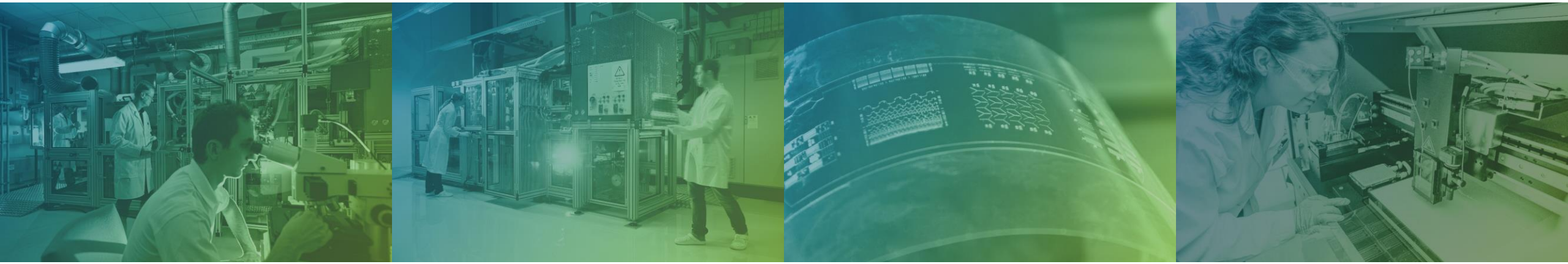
Towards Integration



Integration – what

- Major functionalities required for molecular assay:

	Pumping	Valving	Heating
Reagent storage		X	
Lysis	(X)		X
Purification	X	X	
Amplification	(X)	(X)	X
Detektion/Hybridization	(X) in case of hybridization	(X) in case of hybridization	X

→ All of them can be either integrated in a disposable, or provided by the instrument (or as external process step)

Integration – why

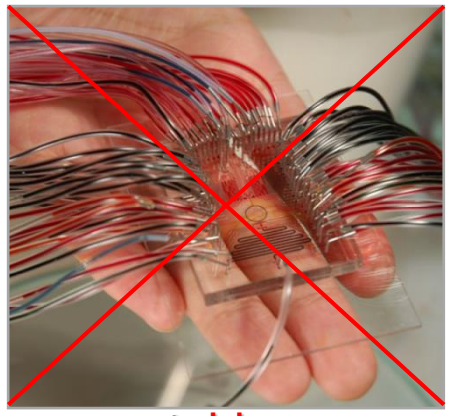
- Low vs. high level of integration:

	Low level of integration (Functions performed by instrument)	**High level of integration** (Functions performed by „disposable“)
Advantages	- Cheaper disposable	- Simple control of disposable - Simpler instrument (or even just a mobile phone), as no mechanical / pneumatic interface needed in instrument - Less maintanance of instrument
Disadvantages	- More complex instrument	- Disposable more costly

Our approach to „full integration“

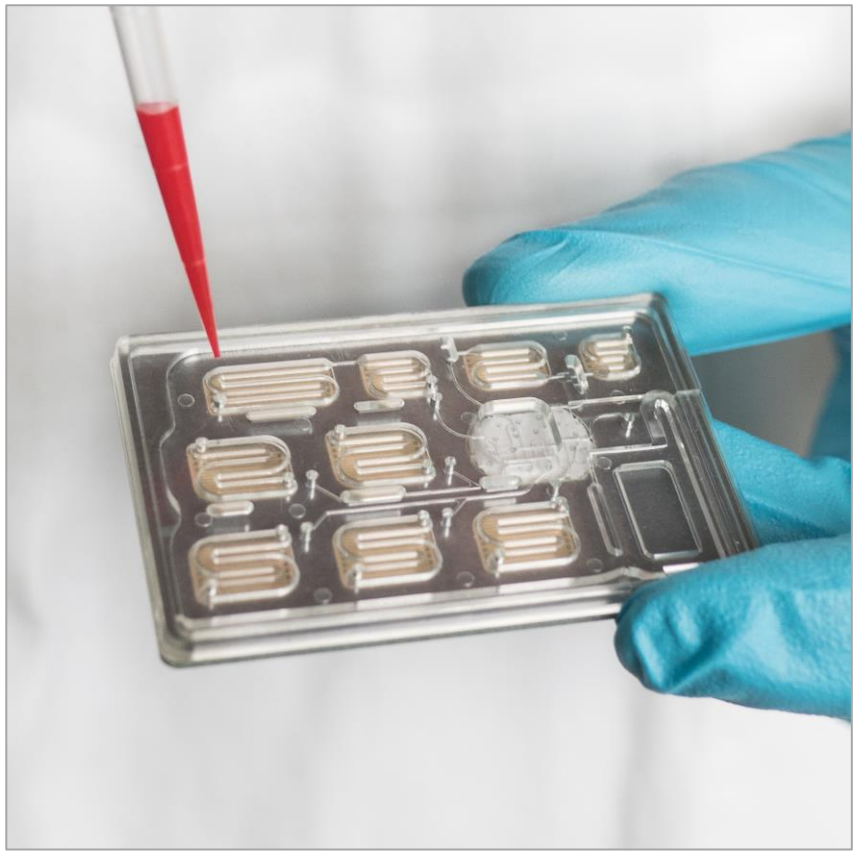
Flexible Microfluidic Platforms
not requiring any tubing, external pumps or external heaters

- ✓ Integrated reagents, micropumps & heaters
- ✓ Low-Cost polymer Lab-on-Chip platforms
- ✓ Standard products ready for evaluation



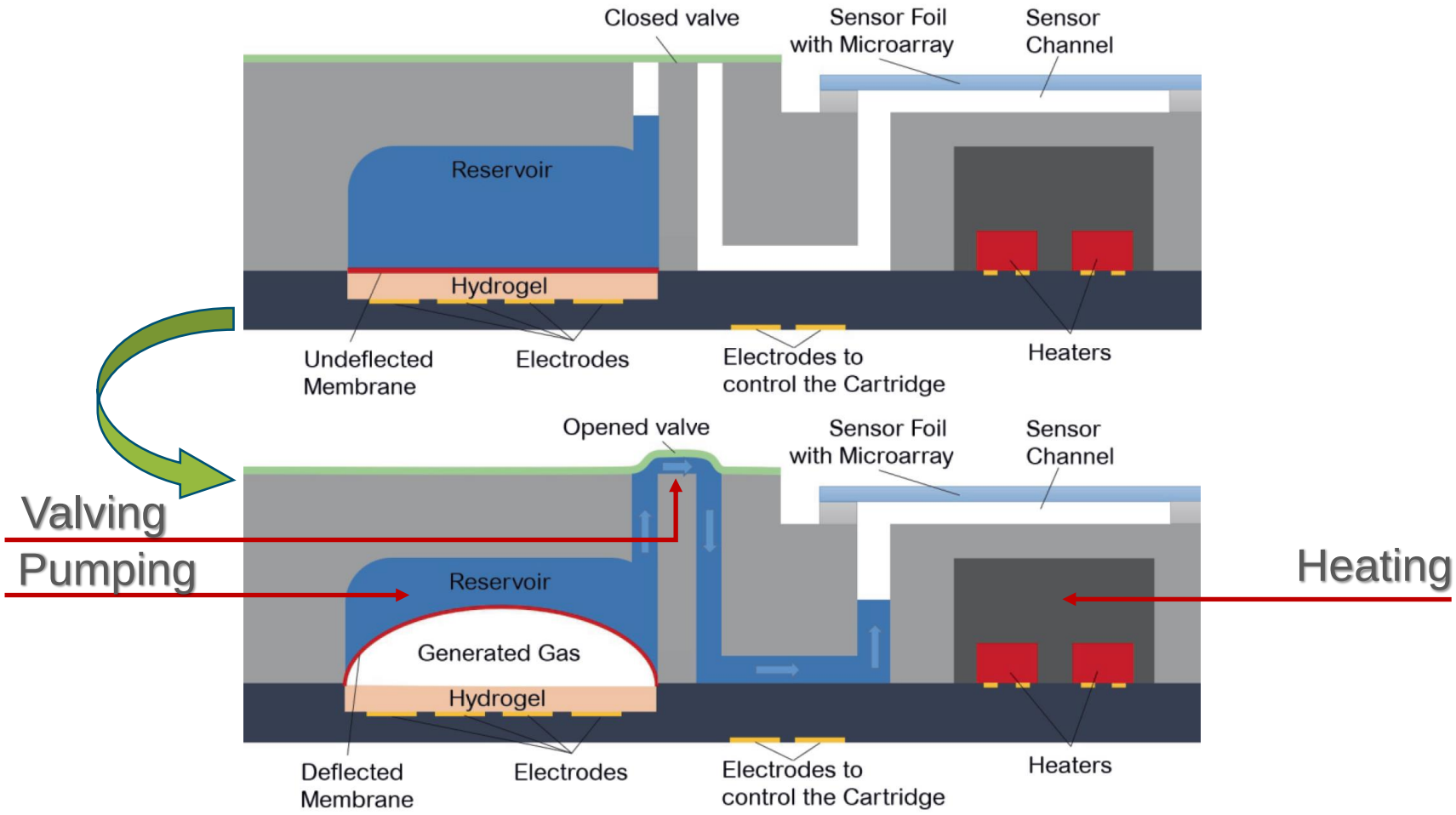
no tubing

no external pumps



On-Chip pumps + reagents

Technology behind...

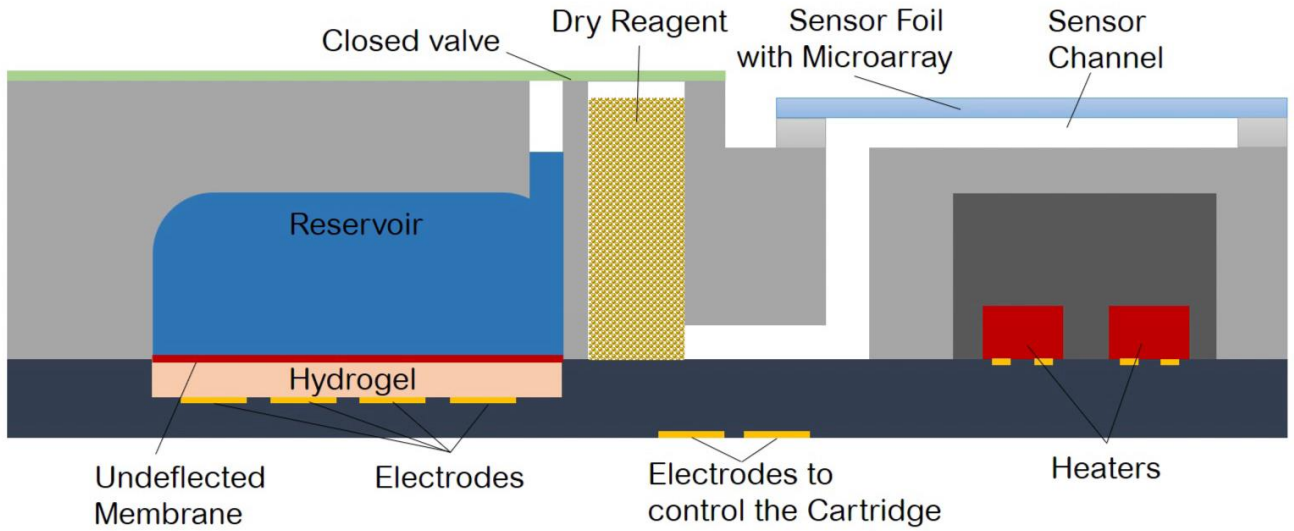


Technology in use: pumping & heating

Video

https://youtu.be/vhli67sttfE

Technology in use: dry reagents

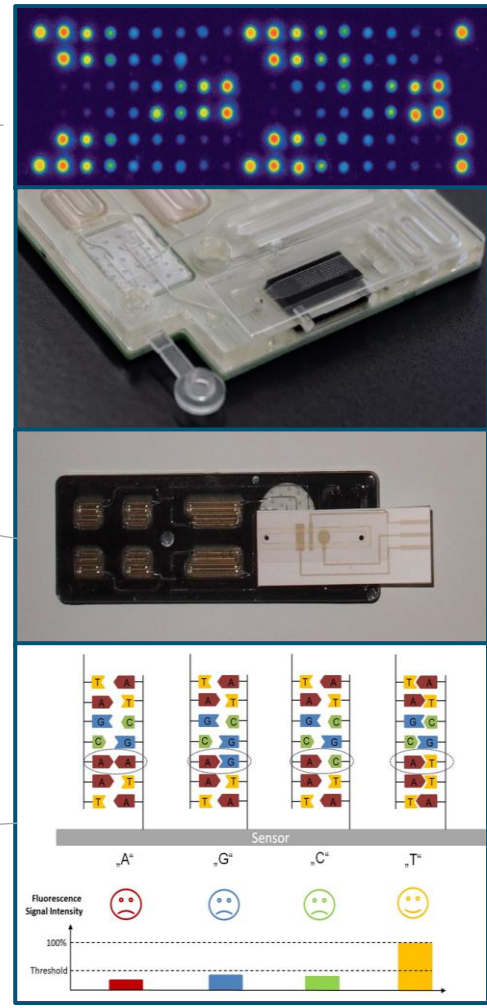


Dry reagents can be integrated using a porous matrix.
The release buffer is stored as described before as liquid.

Video: https://youtu.be/vhli67sttfE

Sensors, Assays, Applications…

- Microarray Integration
- Biosensor Integration
  - Photonic Biosensors
  - Electrochemical Biosensors
  - …
- Assay Integration
  - Immunoassays
  - DNA / RNA-based assays
  - Bead-based assays
  - …
- Examples
  - ELISA-like assays (antigen/antibody detection)
  - DNA-based bacterial species detection
  - DNA-based antibiotic resistance detection
  - RNA-based detection by „new“ integrated amplification method



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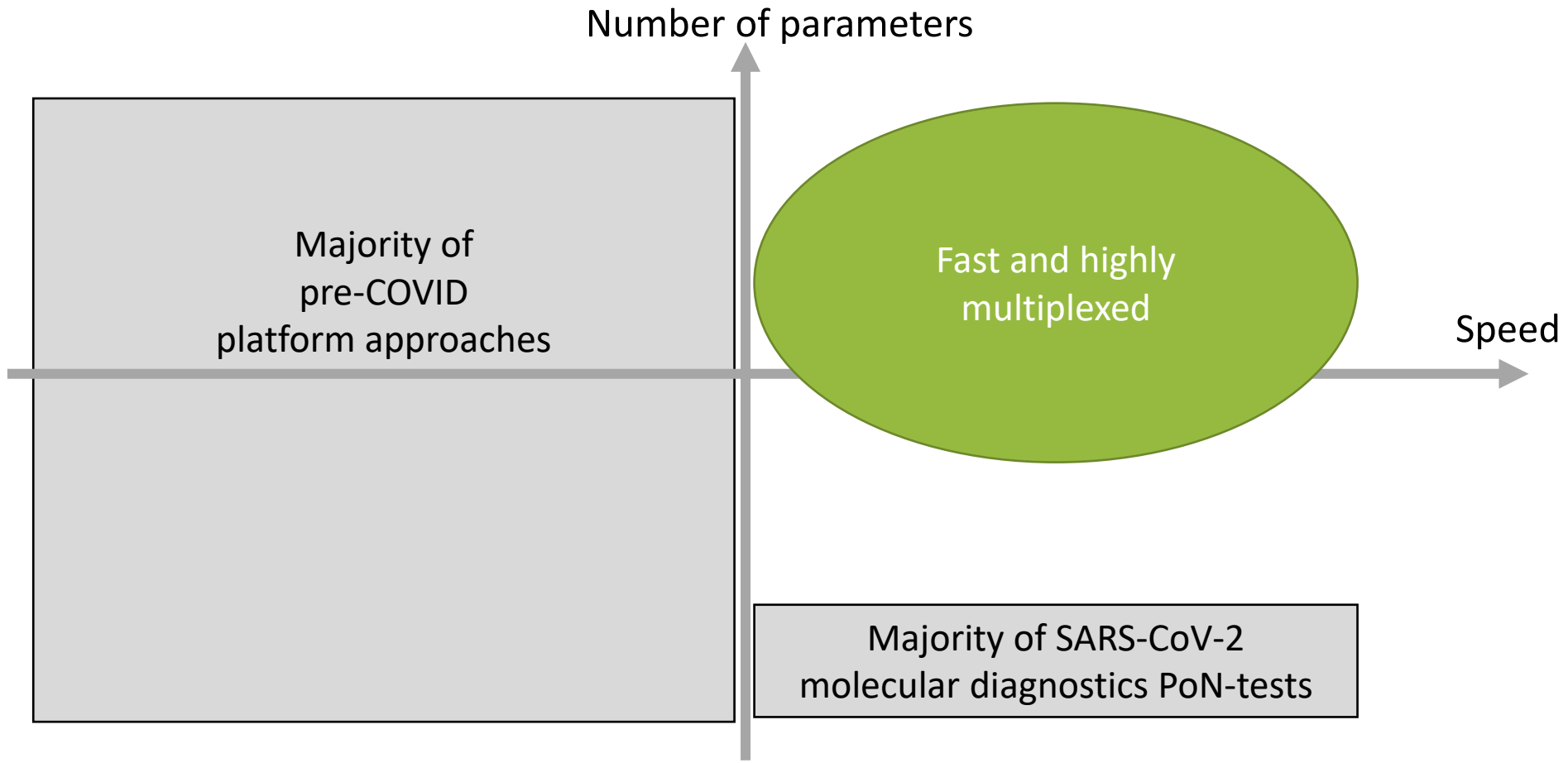
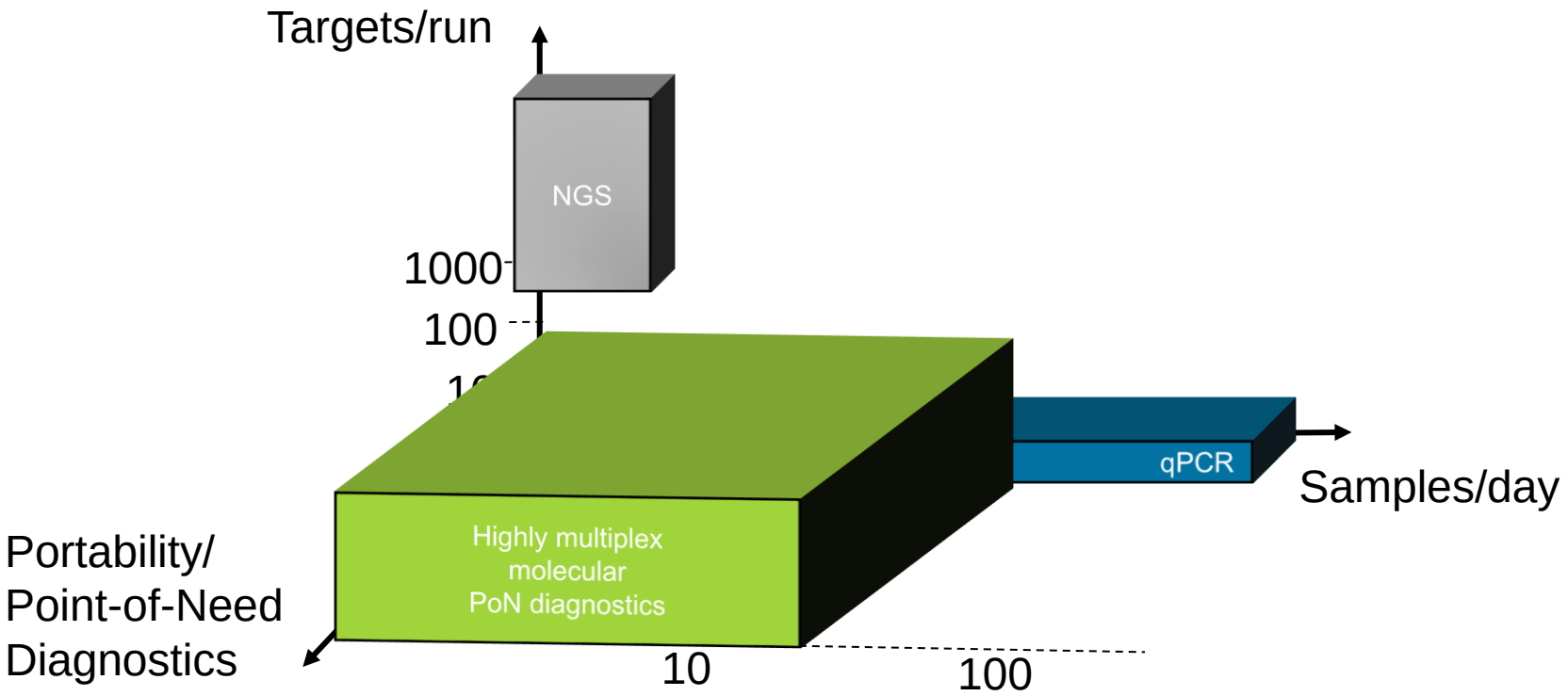
Towards Multiplexing

Multiplex molecular point-of-need diagnostics



Multiplex molecular point-of-need diagnostics



Multiplexing - Why

- **Certain applications require a larger number of parameters, e.g.**
  - Identification / differentiation of
    - Bacterial species
    - Fungee
    - Virus types or their variants
    - Animal and plant species
  - (genotypic) drug resistance testing (especially if for different species)
  - …

Multiplexing – Ways to do

- **During amplification**
  - multiplex qPCR or quantitative isothermal amplification – **single well**
    - Limited, typically not more than 6-plex
    - Limited for most isothermal methods
  - singleplex qPCR or end-point PCR or isotherm. amplif. – **multiple wells**
    - Not limited and possible also for all isothermal methods, however addressing multiple wells limits multiplexing during integration
- **During detection**
  - spacial resolution of different probes, e.g. by specific hybridization on microarray (optical, electrochemical, …)
- **Combination of both**

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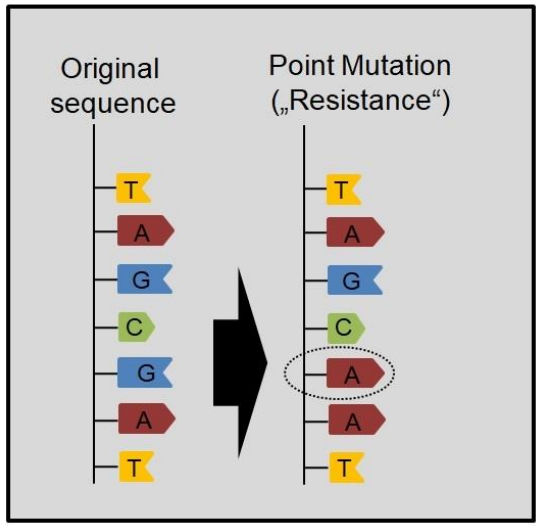
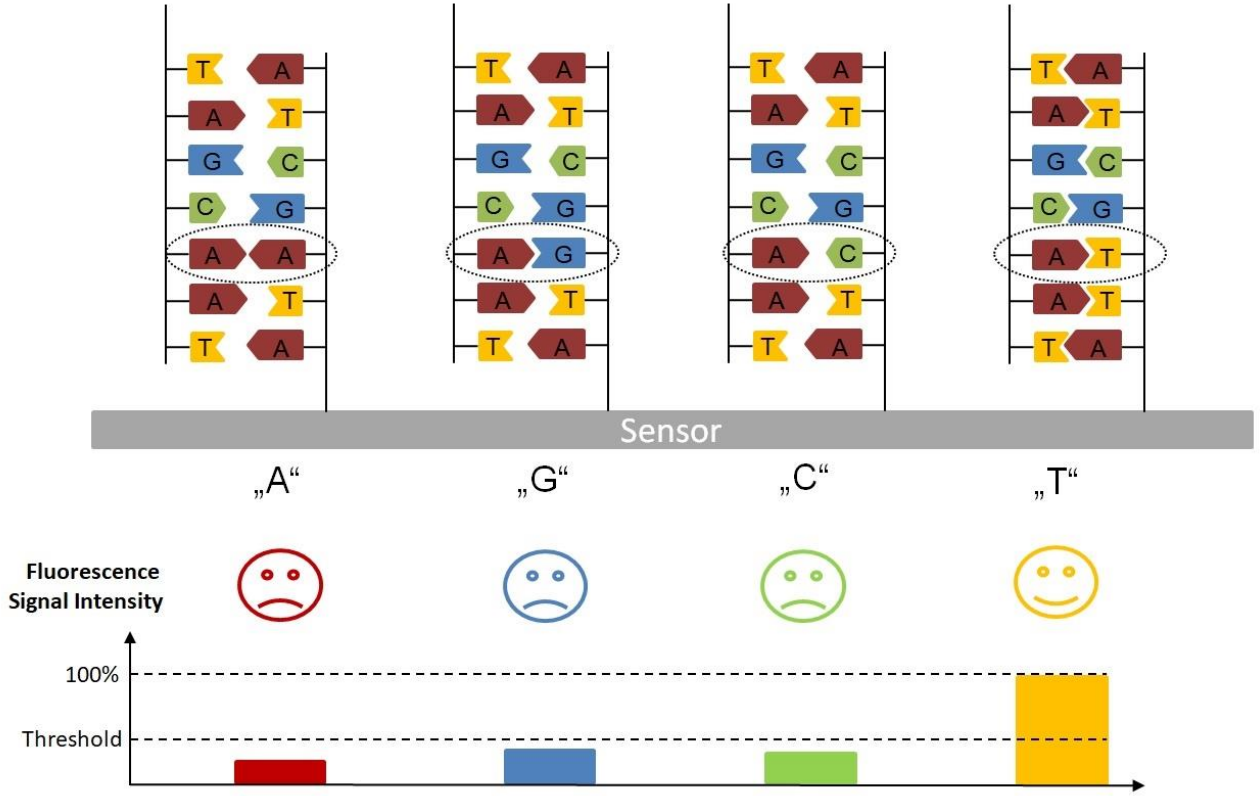
Speed
(an example)

About SNP detection





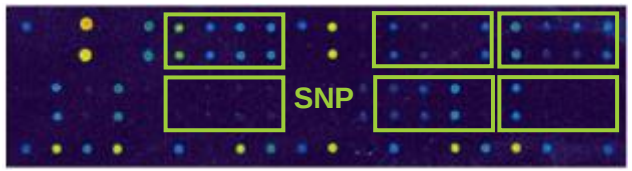
J. Nestler, H. Peter, F.F. Bier, Optik&Photonik 13, 2 (2018), 28-31

Hybridization speed

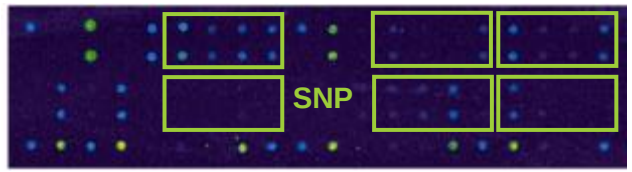
MANUAL Hybridization in Laboratory
120min (lots of labour work)

AUTOMATIC Hybridization in Cartridge

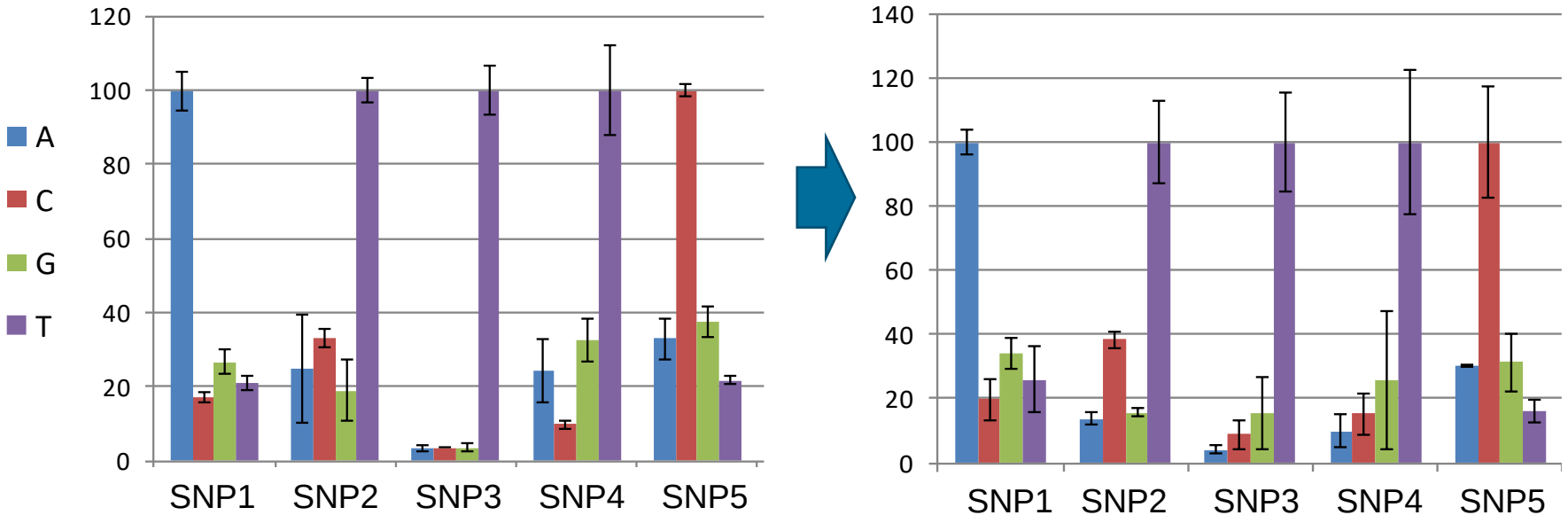
60min



15min



False-color fluorescence image of microarray



Normalized Intensities of SNP probes

Fraunhofer IZI-BB

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bi.FLOW systems GmbH biofluidic integration

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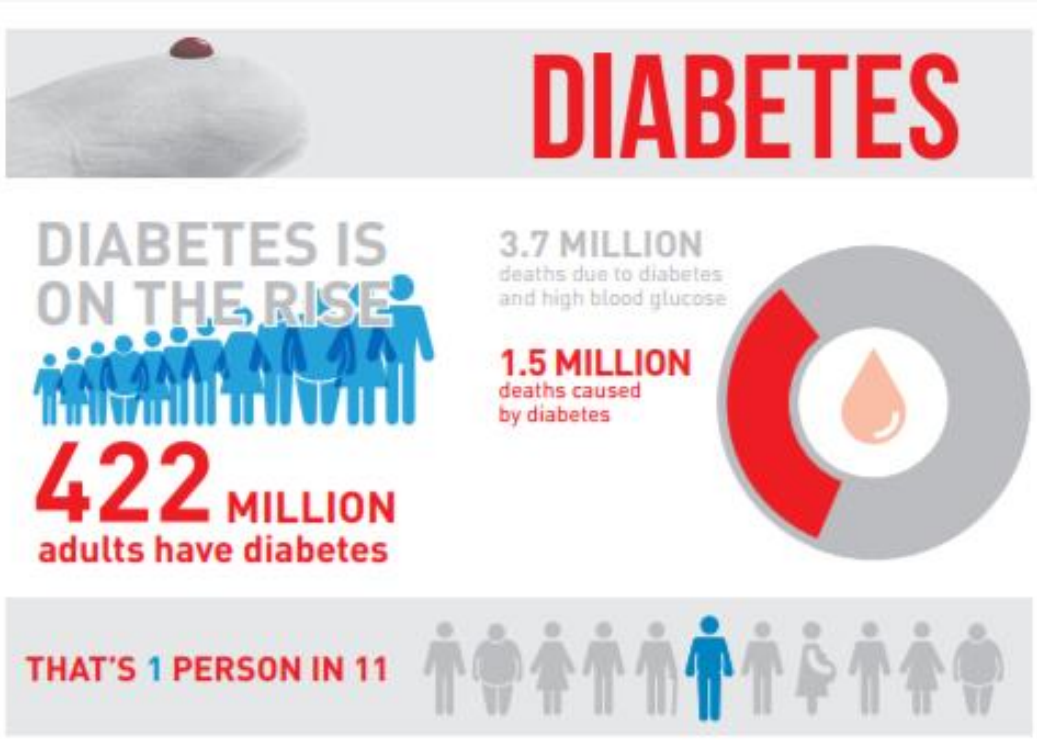
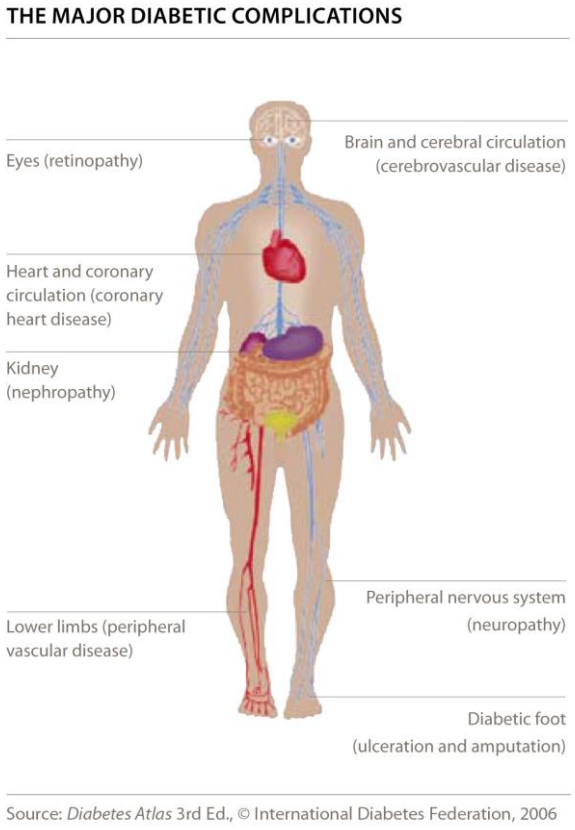
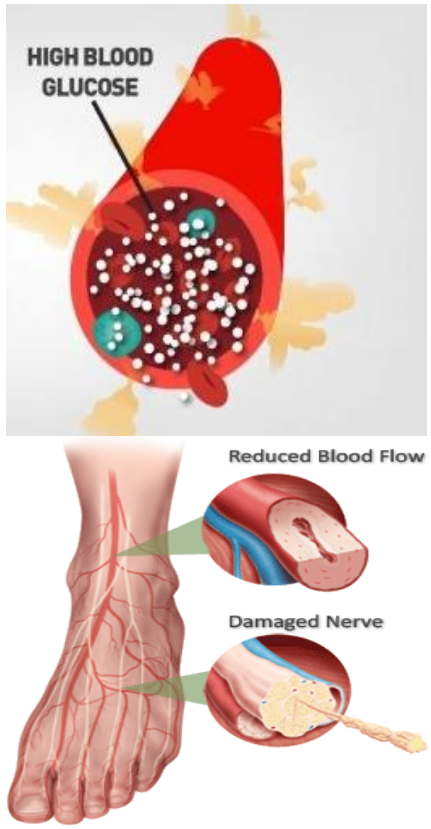
Application: Bacterial species & AMR (for diabetic foot ulcers)

Diabetic foot ulcer



WHO: fact sheet on Diabetes (2018)

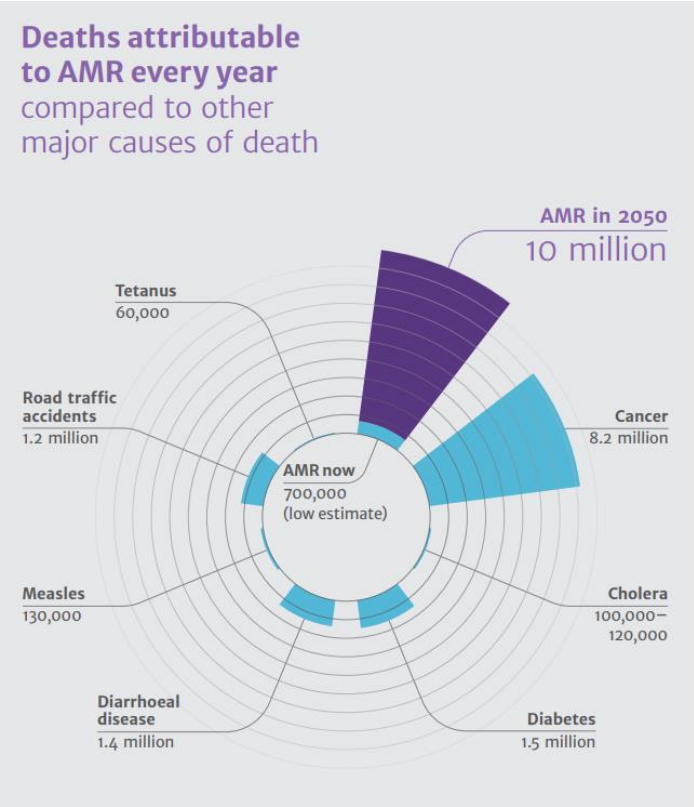




Prevalence of Diabetic foot ulcer world-wide
Global average: 6.3%; Europe: 5.1%
India: 11.6%; Germany 2.8%

(commonswikimedia.org)

Antimicrobial Resistances

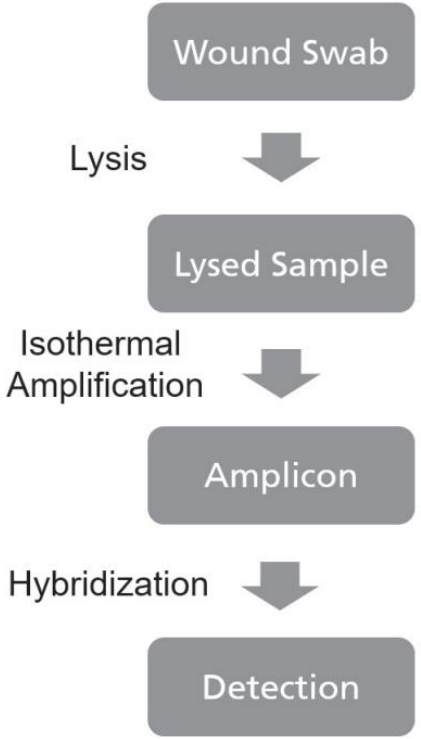


https://www.weforum.org/agenda/2018/03/india-s-war-on-antimicrobial-resistance

Img src: https://kangarama.com/pages/eskape

↑ drug resistance + ↓ novel antibiotic R&D = The end of the "Antibiotic Era"

System Workflow

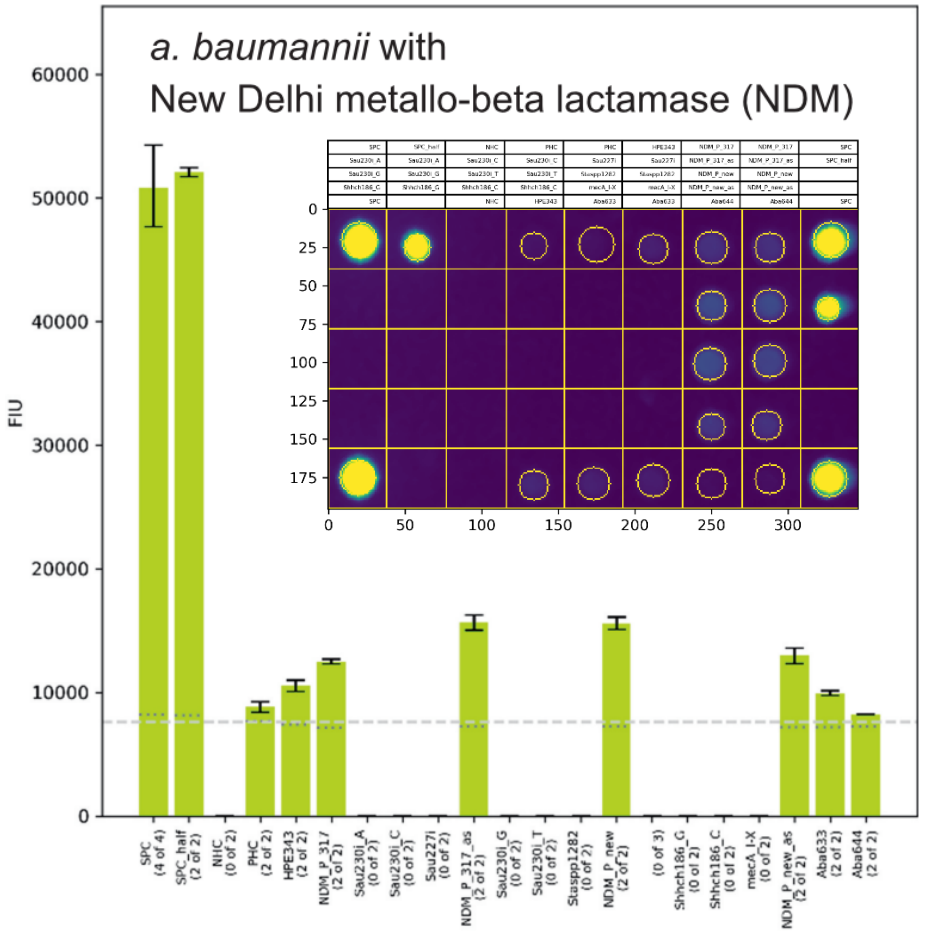


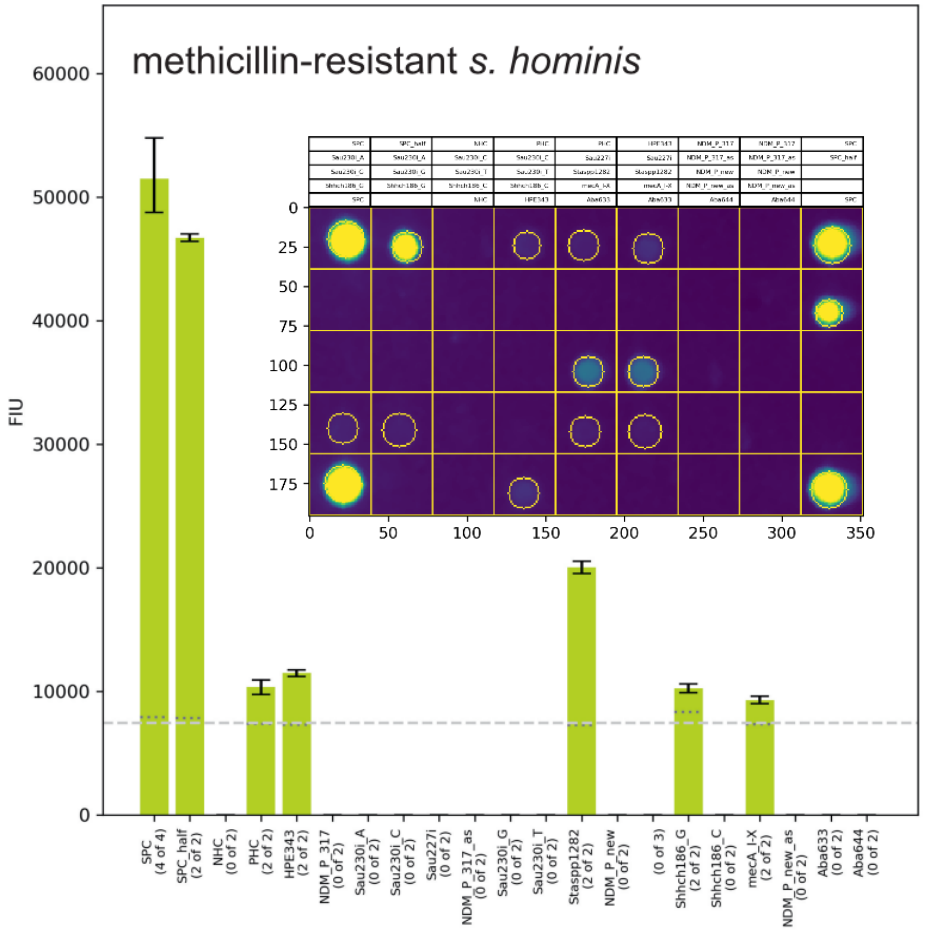
Reader to control and read the cartridge

Successfully demonstrated
combining Microarray + Cartridge + Reader:

- ✓ Amplification on Cartridge in Reader
- ✓ Hybridization on Cartridge in Reader
- ✓ Microarray Readout in Reader
- ✓ Successful detection of different pathogens and resistances

Example Results (Cartridge + Instrument)





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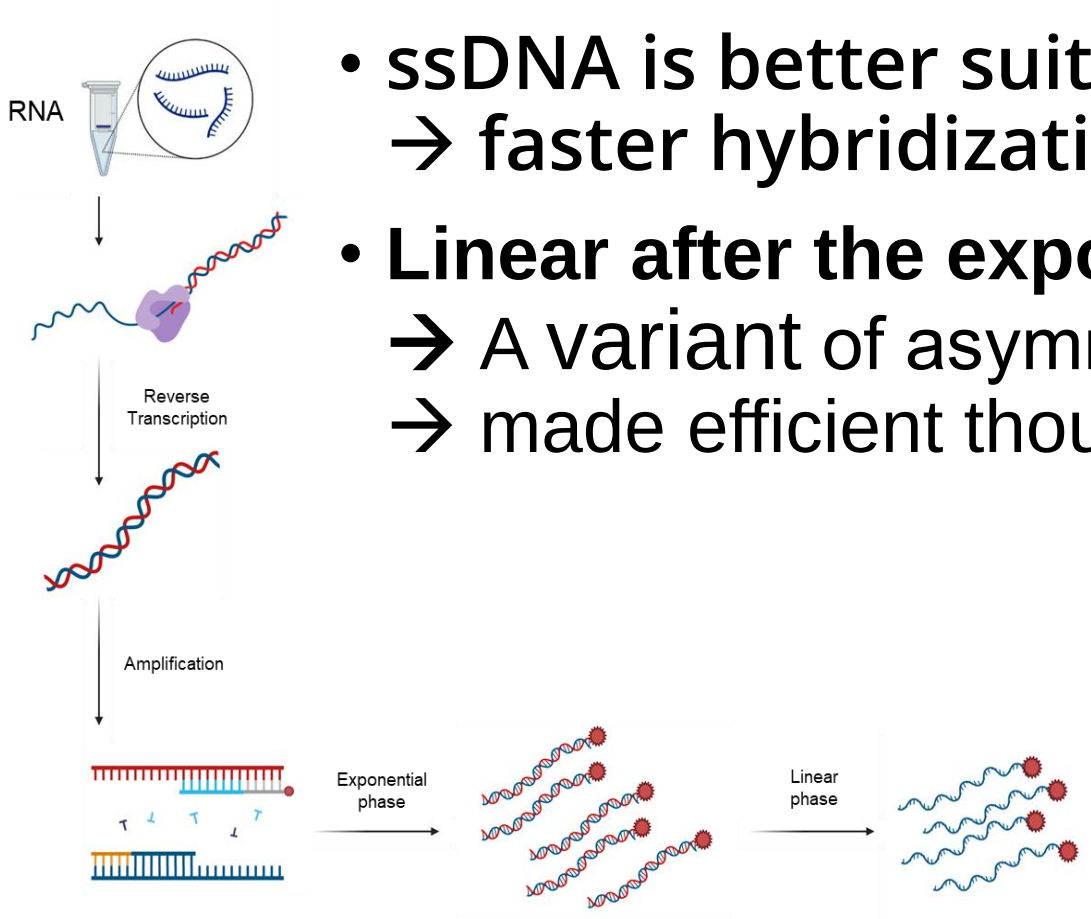
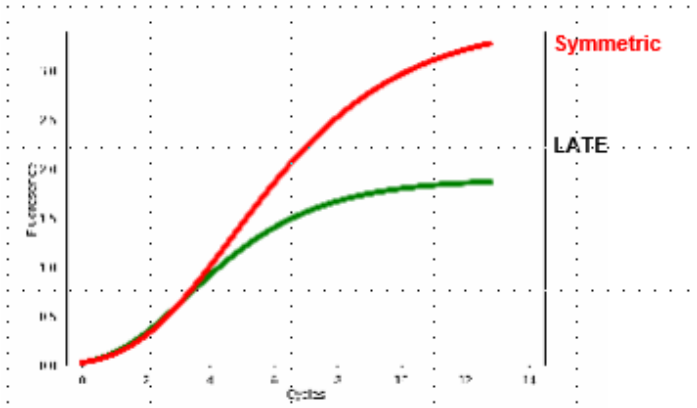
Application: SARS-CoV-2 or Influenza?

ssDNA vs. dsDNA for hybridization?



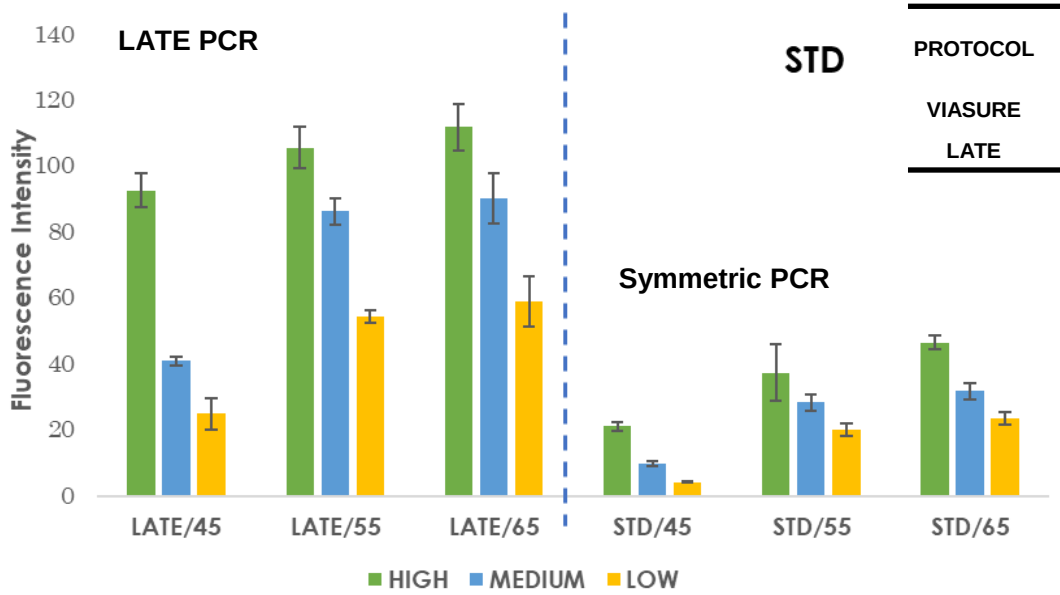
- ssDNA is better suited for hybridization on microarray
  → faster hybridization
- **Linear after the exponential (LATE) PCR**
  → A variant of asymmetric PCR…
  → made efficient though primer modification

Generation of ssDNA in excess



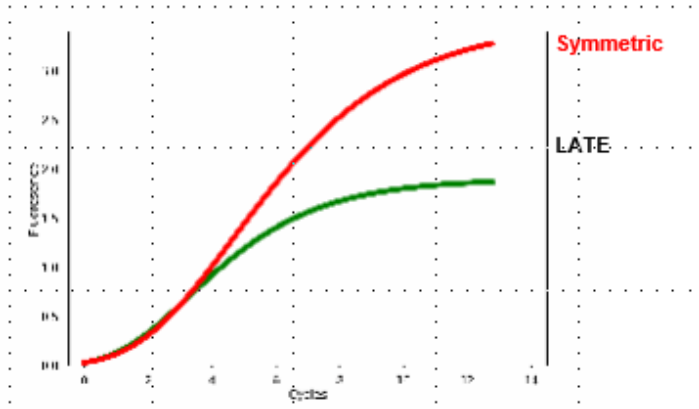
ssDNA vs. dsDNA for hybridization?

- Hybridization results for LATE PCR vs. standard PCR



PROTOCOL	VIRAL LOAD		
	LOW	MEDIUM	HIGH
VIASURE	34.1	24.6	19.7
LATE	31.7	22.3	18.3

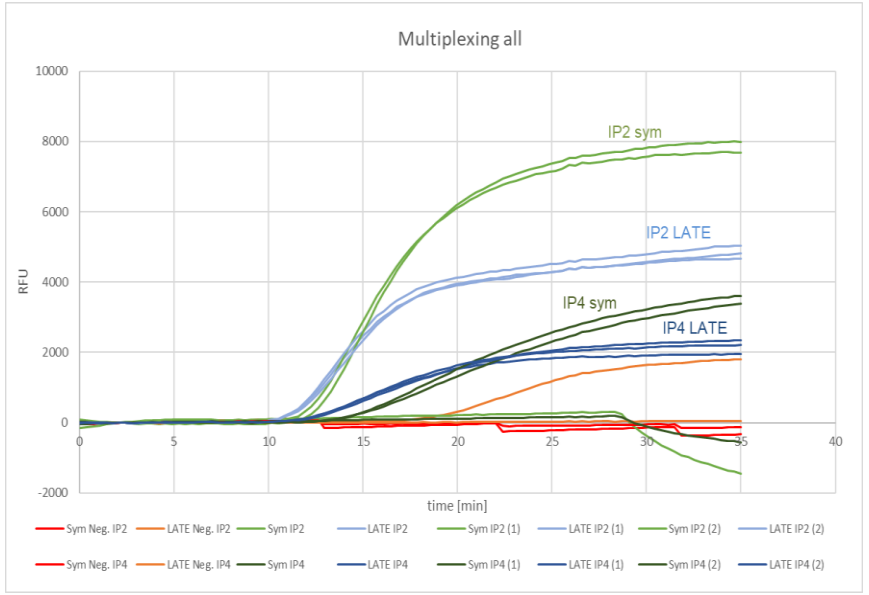
Amplification of the IP2 gene of SARS-CoV-2 in real patient samples and subsequent hybridization of the amplicon with immobilized probes



From LATE-PCR to LATE psiPCR

**Conventional thermocycler (left)
vs pseudo-isothermal PCR cycler (right)
(1500bp: 2hours vs. 30min)**

Multiplexed LATE psiPCR (1000 copies of artificial RNA)



- ✓ **Reduction in time-to-result & power consumption**
- ✓ **Simplification of cartridge design**

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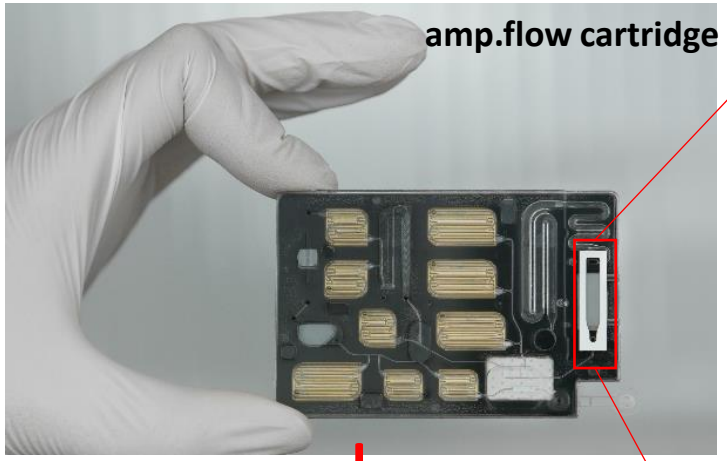
Hybridization cartridge



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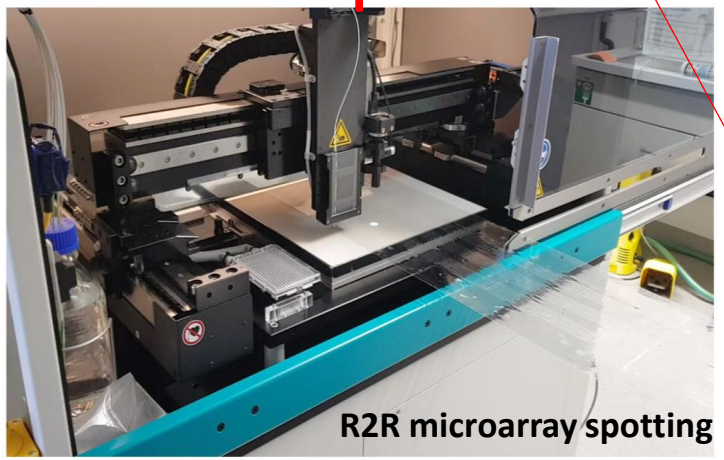

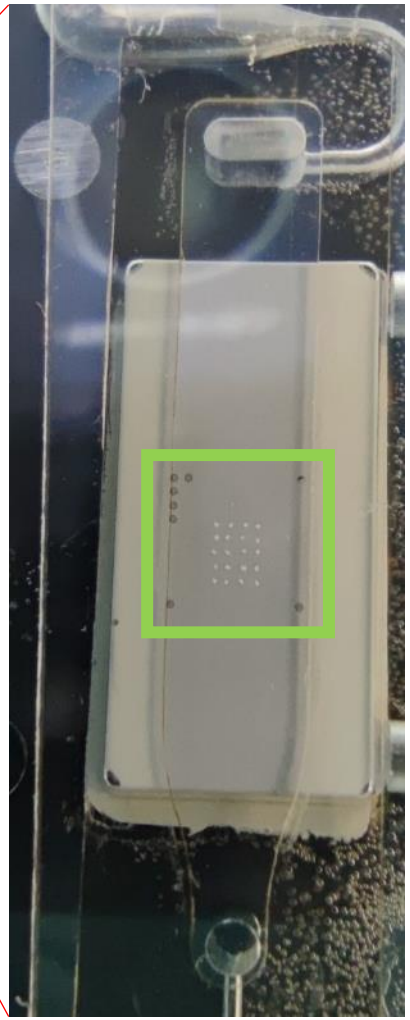

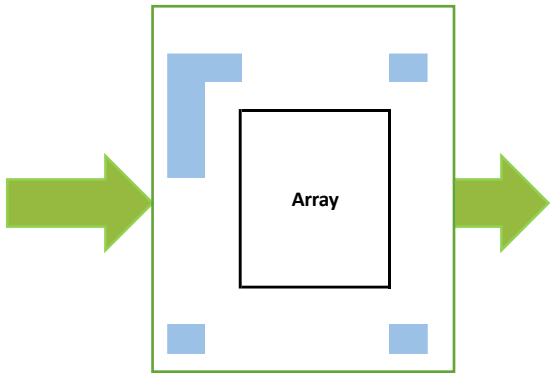

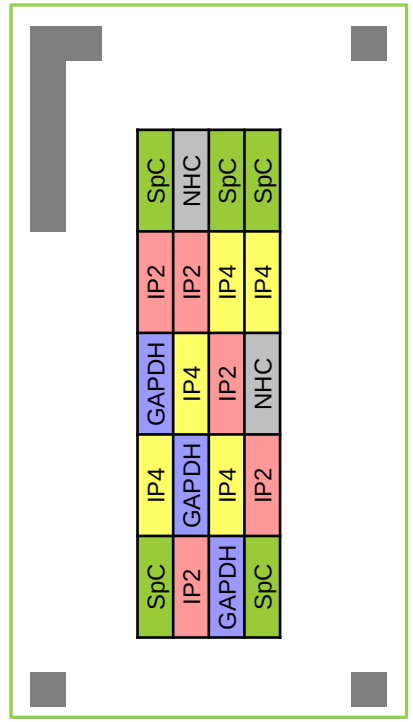

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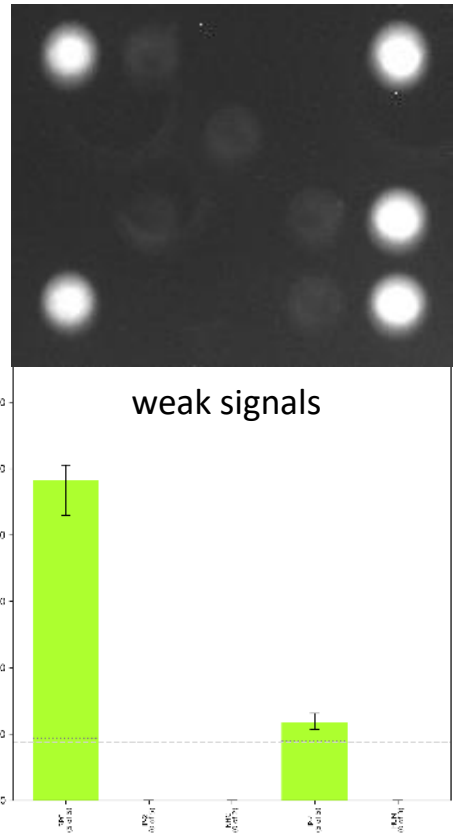
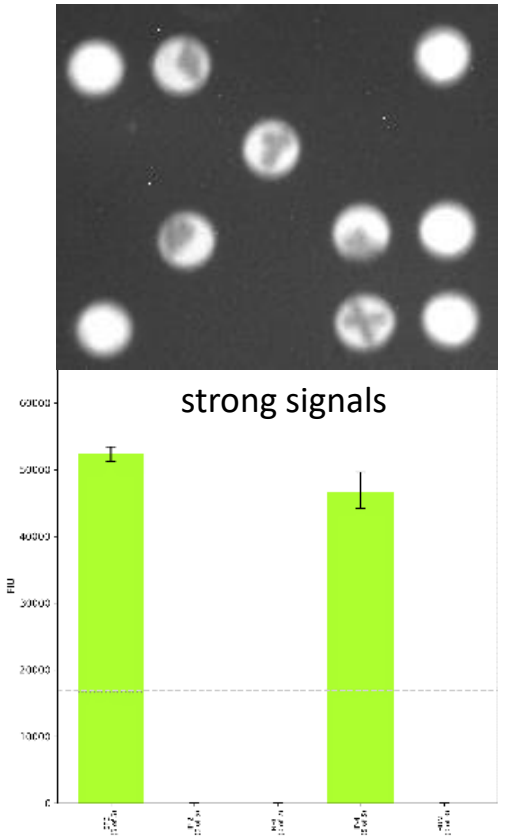
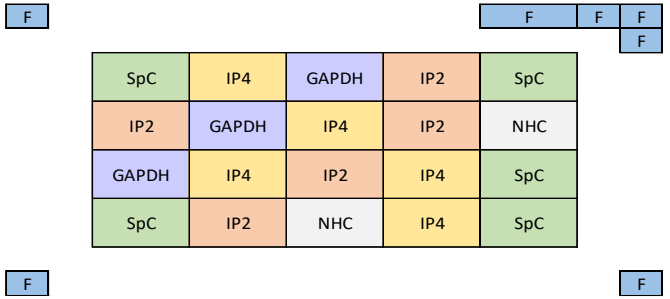
Symmetric psiPCR vs. LATE psiPCR: Hybridization results

Symmetric



LATE psiPCR





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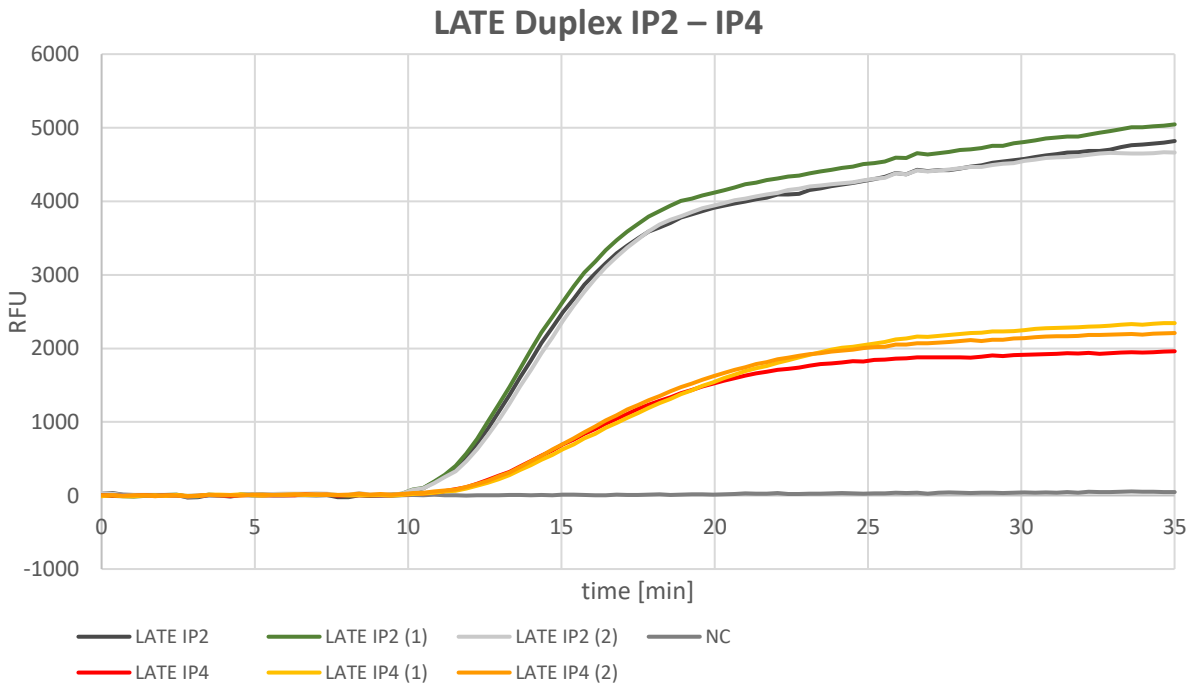
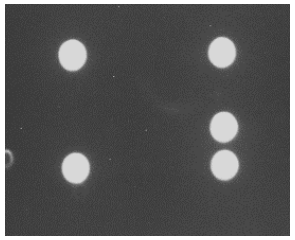
Duplex LATE q-psiPCR, patient sample

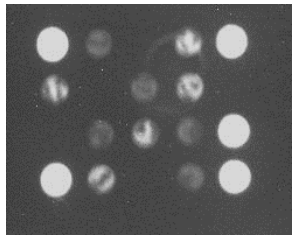


→ Now transfer that protocol to endpoint psiPCR for hybridization on microfluidic cartridge

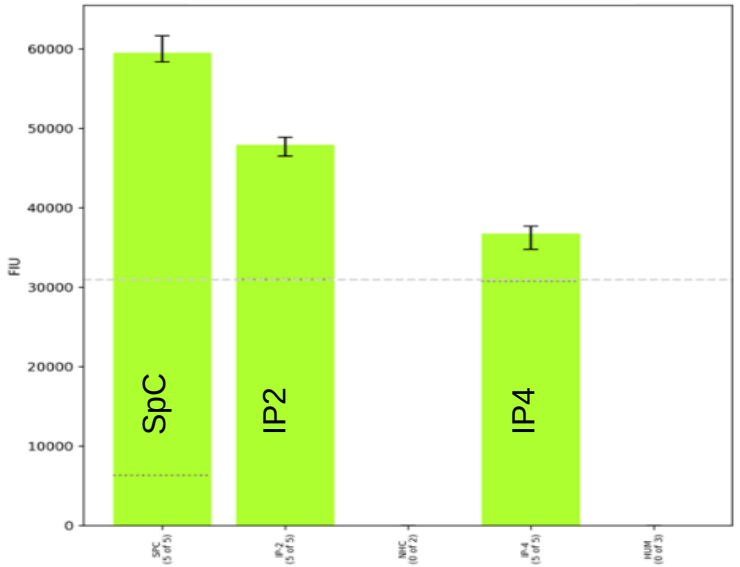
Duplex LATE q-psiPCR, patient sample

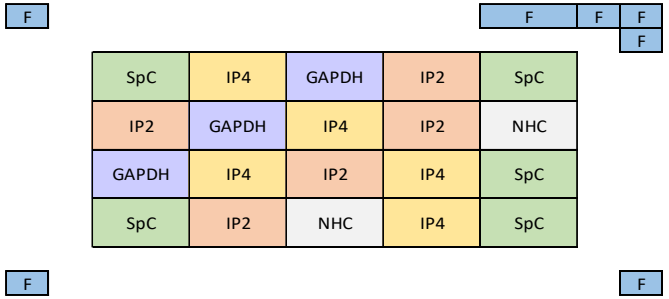


before hybridization



after hybridization





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SUMMARY

Summary

- **Aspects and strategies to consider for Lab-on-a-Chip for molecular diagnostics:**
  - Level of Integration
  - Level of Multiplexing
  - Speed
- **Examples**
  - Bacterial Species Identification & AMR
  - Virus identification

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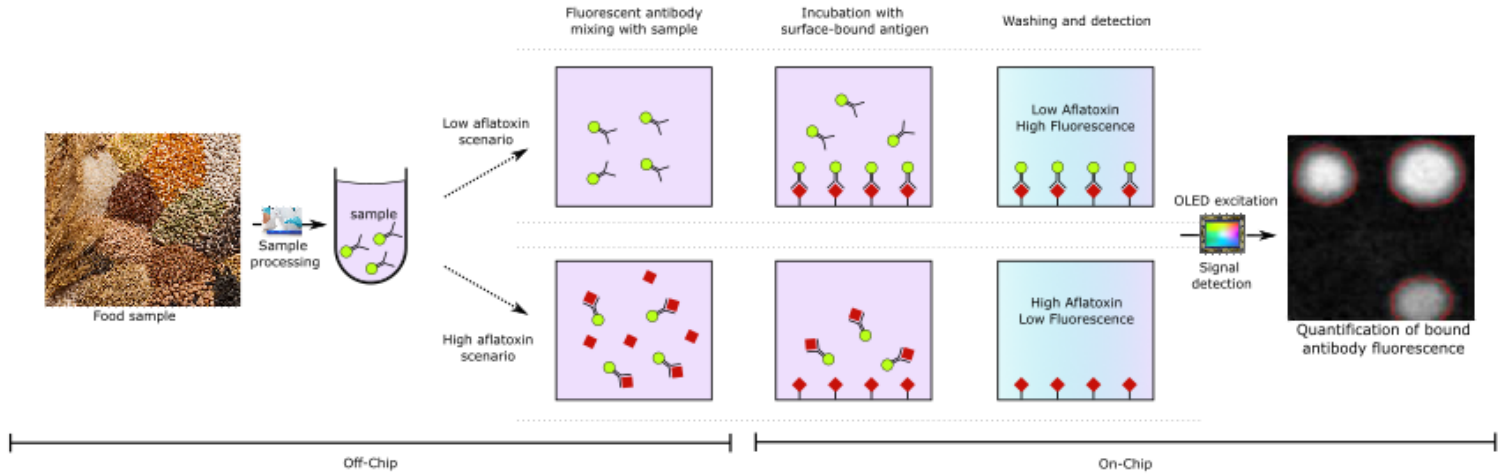
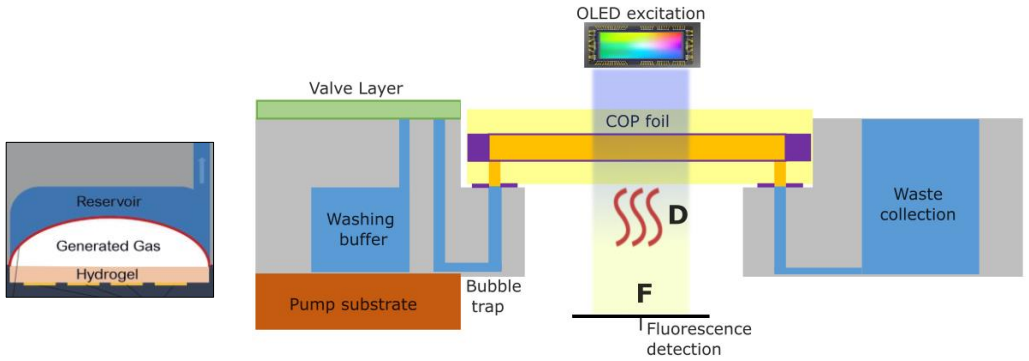
Question Time!

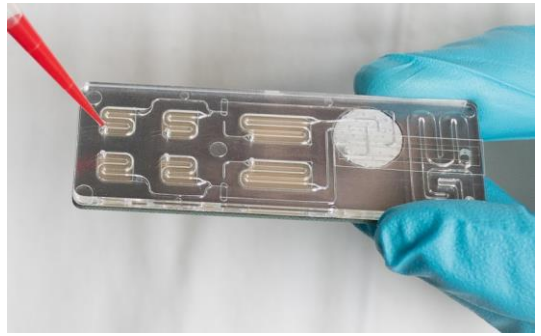
Or-el

Primary Components: Assay (Or-el) | Design (bi.FLOW) | Patterning (bi.FLOW) | Surface functionalisation (ibidi, JOANNEUM RESEARCH) | Electrodes Sensors | Backend Services (bi.FLOW) | Read Out Device System Integration (Or-el, bi.FLOW) | QM (bi.FLOW)

Competitive ELISA for high-sensitivity detection of mycotoxins Aflatoxin A1 / M1







Or-el

Benchtop Readout Device

- OLED excitation and detection technology by Or-El
- Non-expert operation

bi.FLOW systems biofluidic integration

Fully integrated and automated disposable microfluidic Cartridge

- Low-cost
- 6-12 month shelf life
- Integrated liquid reagent storage
- Complete automation with integrated electronics
- Low footprint (standard microscopy slide dimensions)
- Flexibility – multiple toxin detection possible

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Accelerate your Microfluidic Innovation

- Addresses companies – SMEs and LEs
- Access to all services of the NGM OITB - min 2 partners involved
- Budget up to EUR 200.000
- Duration: 6 to 12 months
- Funding rate of up to 92% and 50% for European SMEs and Large Enterprises respectively
- Technology Readiness Level > 4 or Microfluidic System available
- Managed & coordinated by the MIH
- Details see: www.nextgenmicrofluidics.eu/open-call/

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WE DEVELOP AND PRODUCE CUSTOMIZED

Microfluidic Lab-on-a-Foil Systems

- Single entry point to research & development services
- Comprehensive service portfolio
- Fast prototyping and scale up
- Multiple funding opportunities
- Quality assurance

Find out more at https://www.microfluidicshub.eu

Microfluidics Innovation Hub is the single entry point of the European project NextGenMicrofluidcs (www.nextgenmicrofluidics.eu). NextGenMicrofluidics has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.

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WEBINAR: WE GET MICROFLUIDICS ROLLING

Advantages of Roll-to-roll Replication

ANDRÉ BLEISE
Temicon

ANJA HAASE
Joanneum Research

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13 OCTOBER 2022

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