AUSTRIAN Microfluidics Initiative

OPERATED BY BNN

NextGen Microfluidics

Development and Upscaling of Microfluidic Cartridges for LoC Nucleic Acid Amplification and Microarray Detection

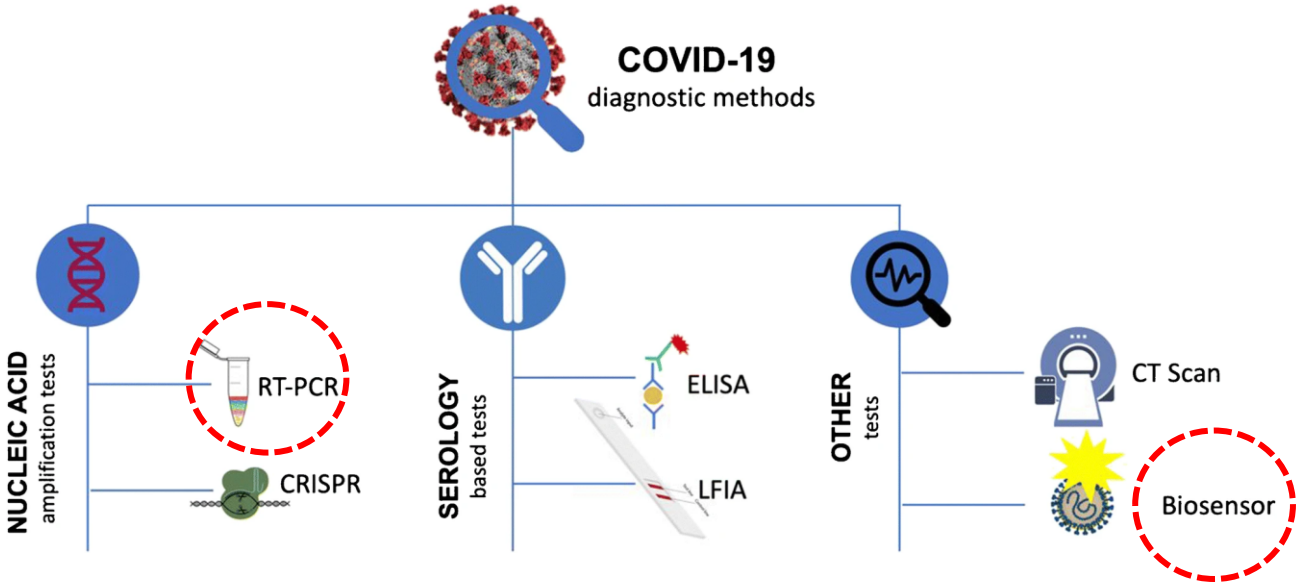
George Tsekenis

Bionanotechnology and Nanochemistry Laboratory,
Biomedical Research Foundation of the Academy of Athens,
Athens, Greece

Societal challenges in COVID-19 crisis

Requirements for **tests that are:**

- **Reliable**
- **Fast**
- **Capable of multiplexed analysis**
- **Cheap**



Our solution

A **Point-of-Care device** that combines:

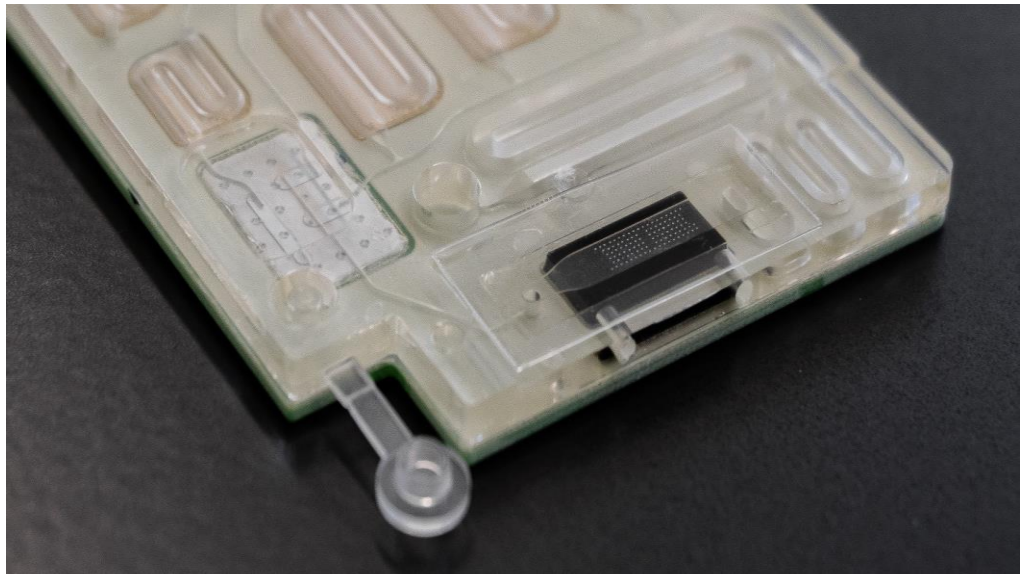
injection molded cartridges

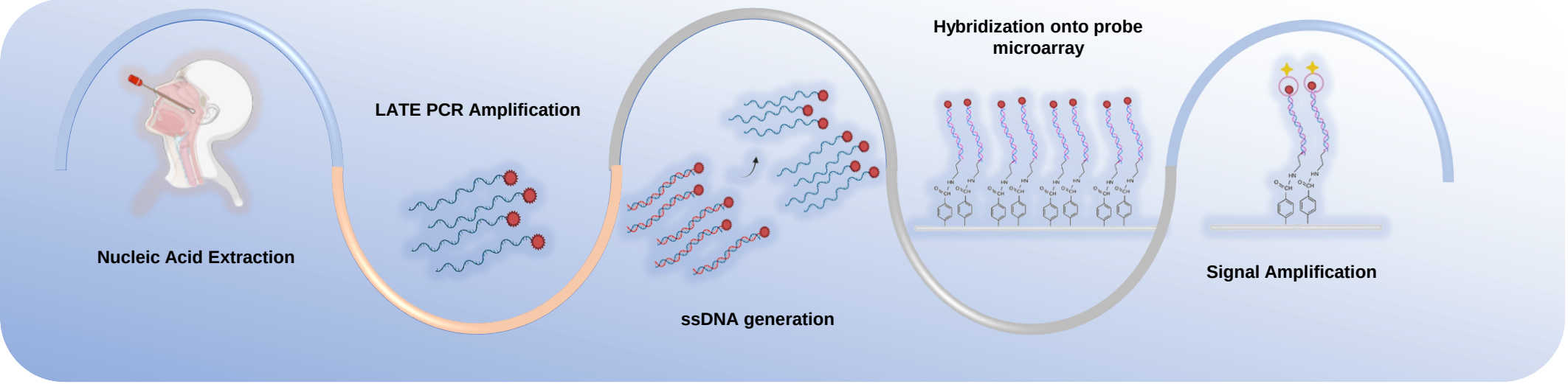
+

sensor foils produced by R2R imprinting

and puts forward innovative solutions in the**:**

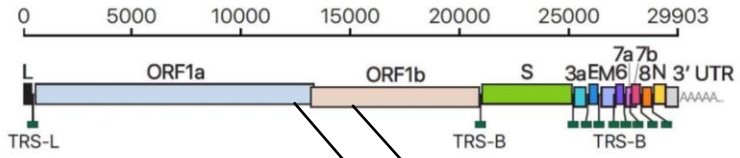
- ✓ **PCR protocol**
- ✓ **Detection methodology**
- ✓ **Integration into a single cartridge**



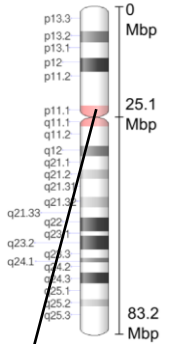


NextGen Microfluidics

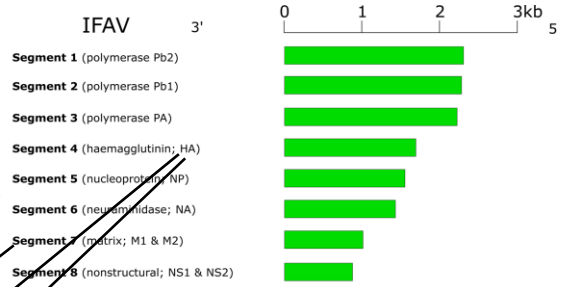
SARS-CoV-2 genome

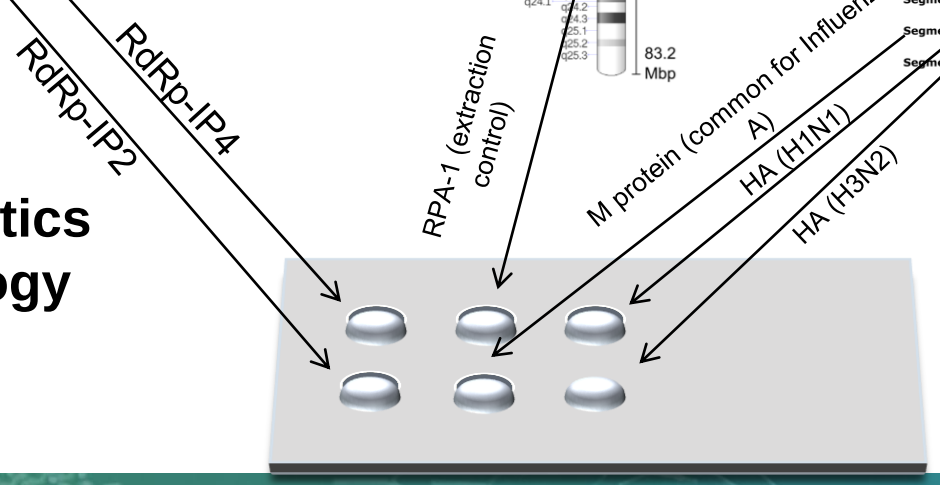


Human chromosome 17



Influenza type A genome



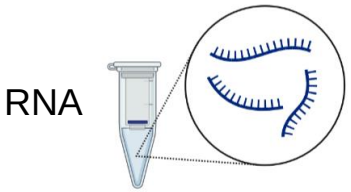


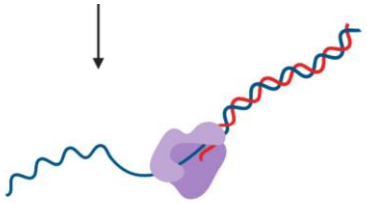
Overview of the molecular diagnostics assay and the detection methodology

✓ **Multiplexed analysis**

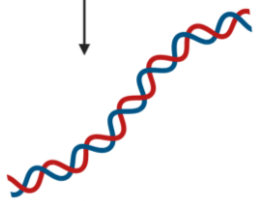
Different immobilized ssDNA probes in every spot

Linear after the exponential (LATE) PCR:
A variant of Asymmetric PCR…made efficient though primer modification

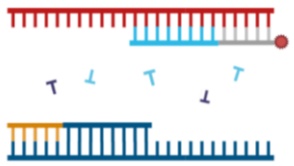




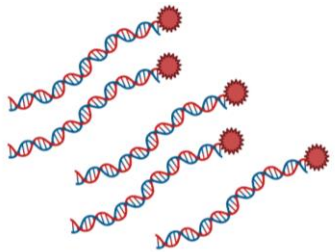
Reverse Transcription



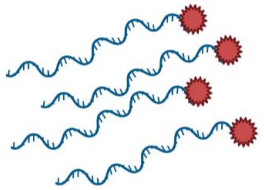
Amplification



Exponential phase



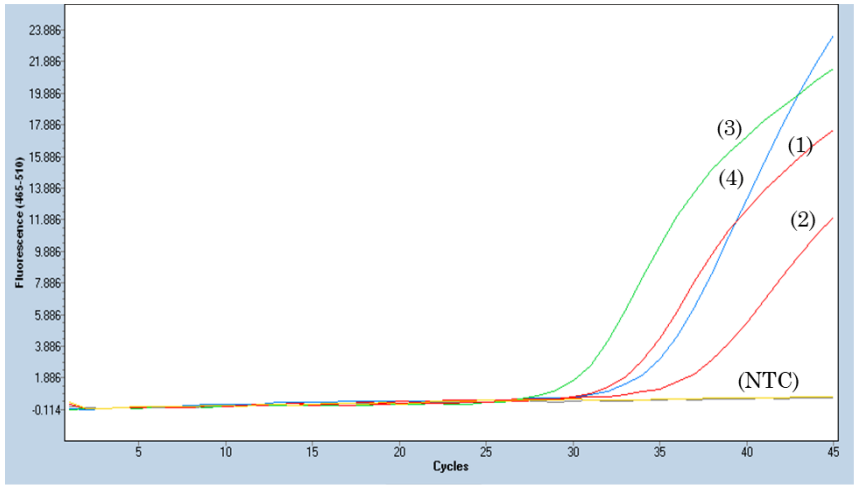
Linear phase



✓ **Generation of ssDNA product in excess**

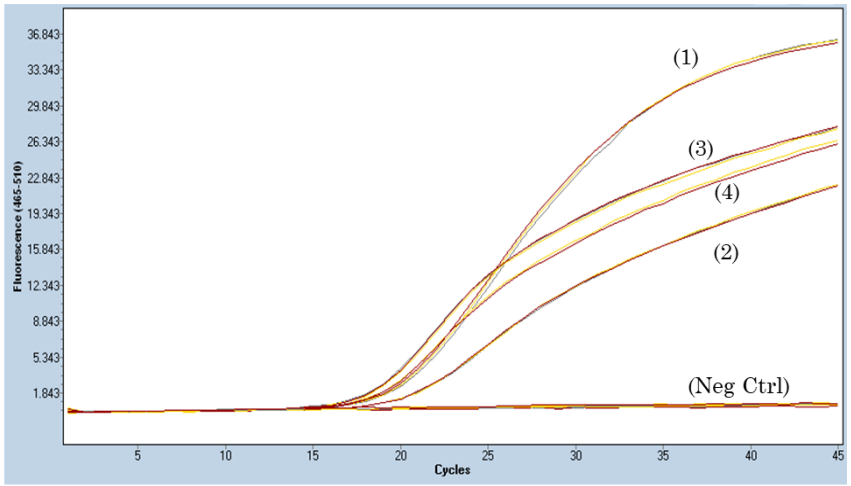
SARS-CoV-2 IP2 gene amplification on conventional benchtop thermocycler

1000 copies of artificial RNA

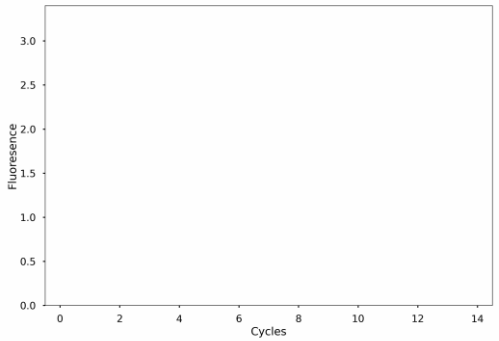


(1) : +Strandard protocol → Cp = 35.09
(2) : no xtra bases → Cp = 37.04
(3) : +2 xtra bases → Cp = 30.32
(4) : +4 xtra bases → Cp = 33.02

Positive Sample



(1) : +Strandard protocol → Cp = 20.19
(2) : no xtra bases → Cp = 20.74
(3) : +2 xtra bases → Cp = 18.29
(4) : +4 xtra bases → Cp = 18.72

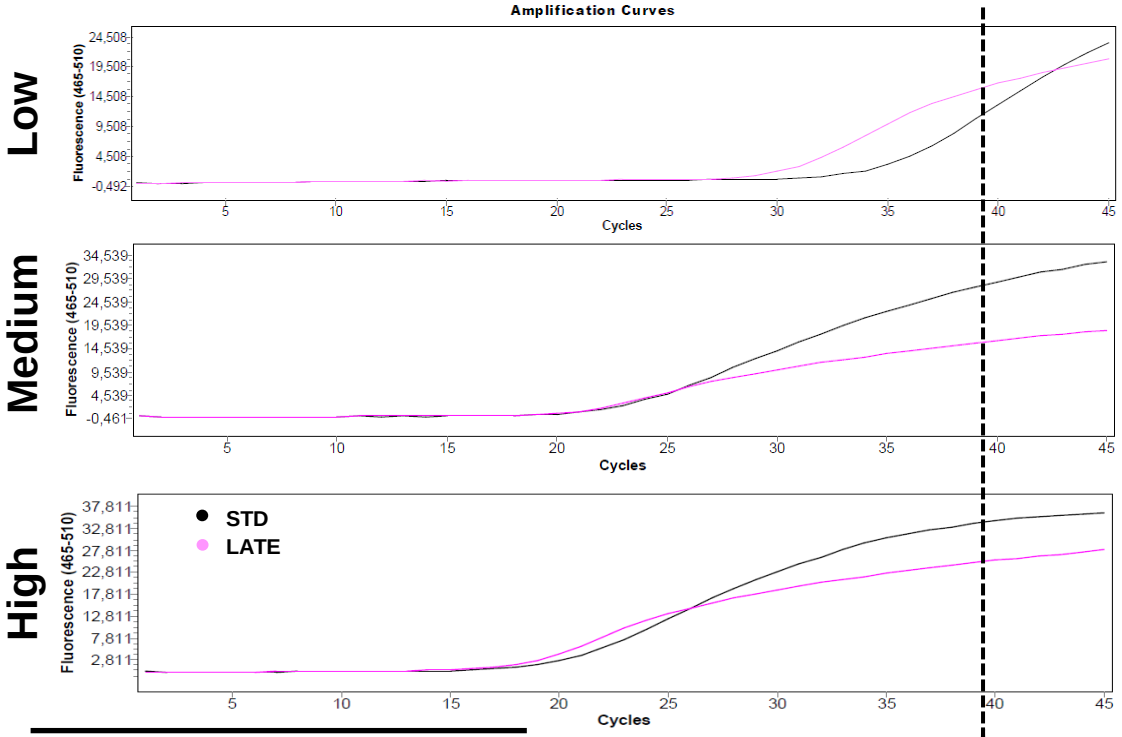


STD

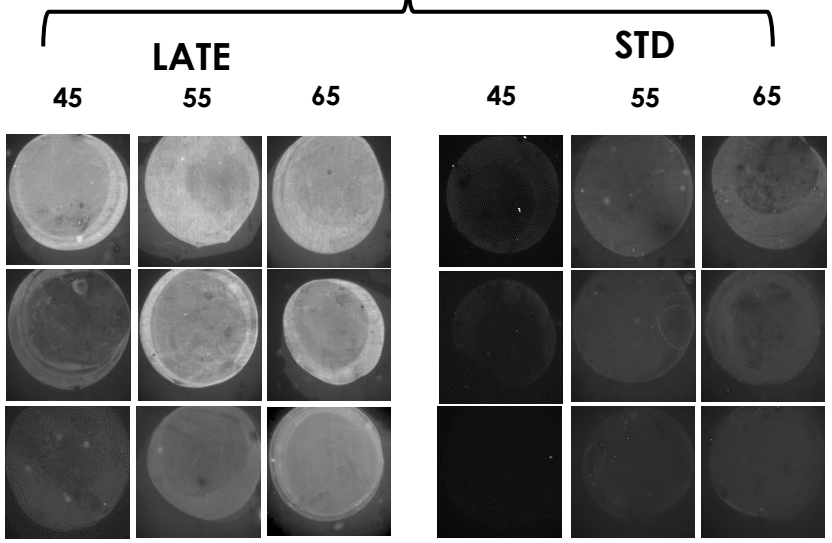
LATE

Detection of amplified product onto probe-spotted sensor foils

Amplification and subsequent detection of patient samples with various viral loads with LATE protocol vs. certified VIASURE kit (STD)



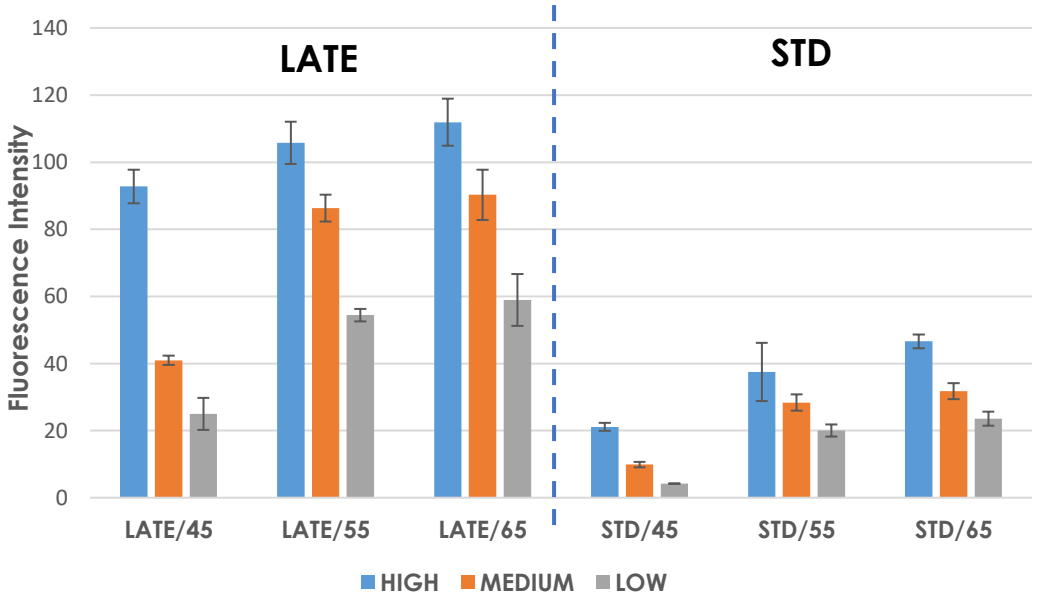
PROTOCOL	VIRAL LOAD		
	LOW	MEDIUM	HIGH
VIASURE	34.1	24.6	19.7
LATE	31.7	22.3	18.3



Hybridization of amplified IP2 with complementary probes spotted onto functionalized COC sensor foils

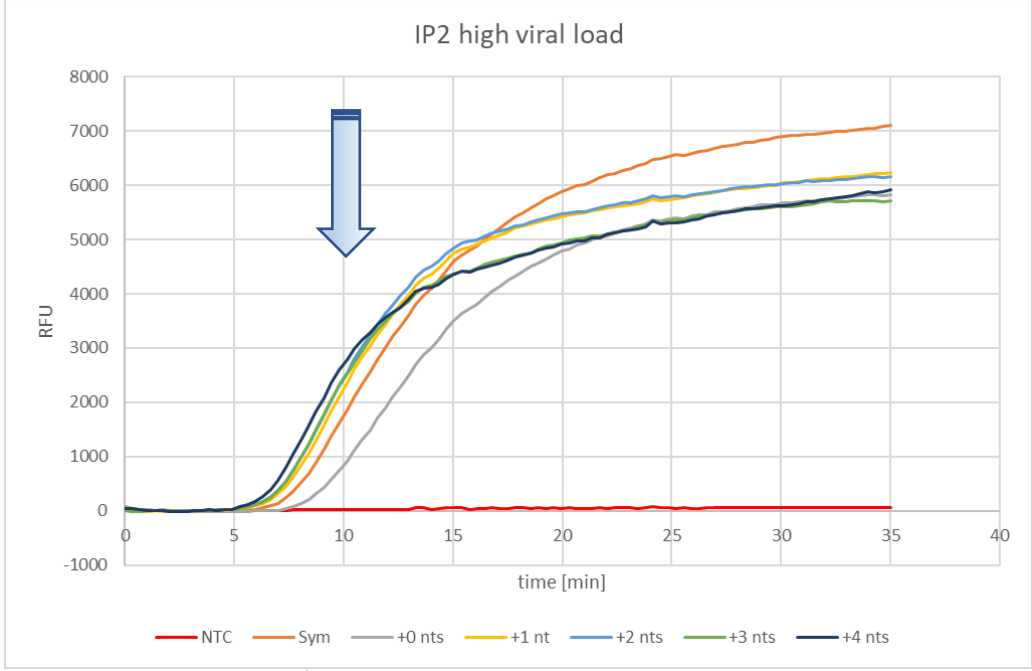
✓ **Faster analysis**

✓ **Simplicity of cartridge design**



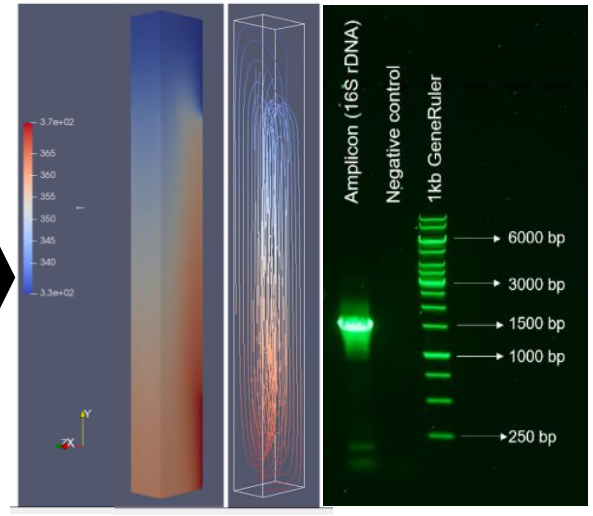
Convective PCR

- ✓ **Further reductions in amplification time**
- ✓ **Further simplification of cartridge design**



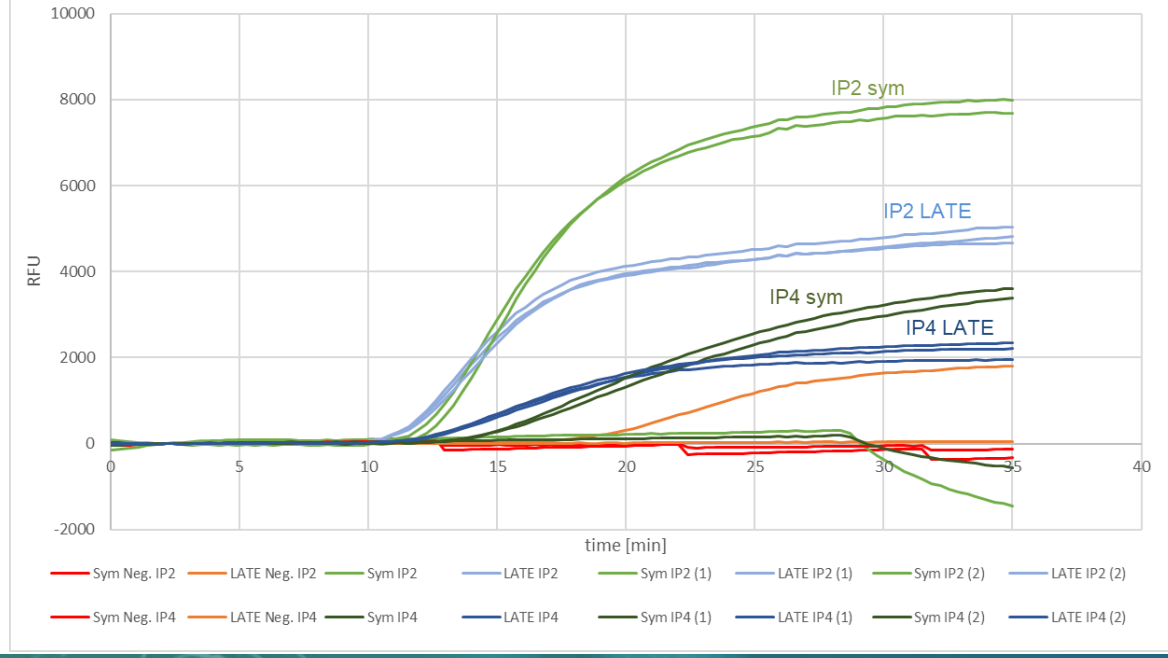
LATE cPCR for IP2 gene (patient sample)

Conventional thermocycler vs cPCR cycler (1500bp: 2hours vs. 30min)



cPCR: Convection Simulation and gel picture of cPCR of 16S rDNA (1500bp) in 30min

Multiplexed LATE cPCR (1000 copies of artificial RNA)



Integration into a cartridge

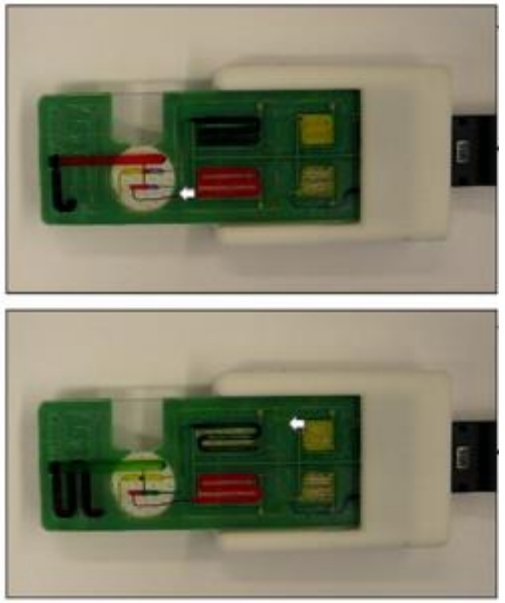
Flexible Microfluidic Platforms not requiring any tubing, external pumps or external heaters

✓ Integrated reagents, micropumps & heaters

no tubing

no external pumps

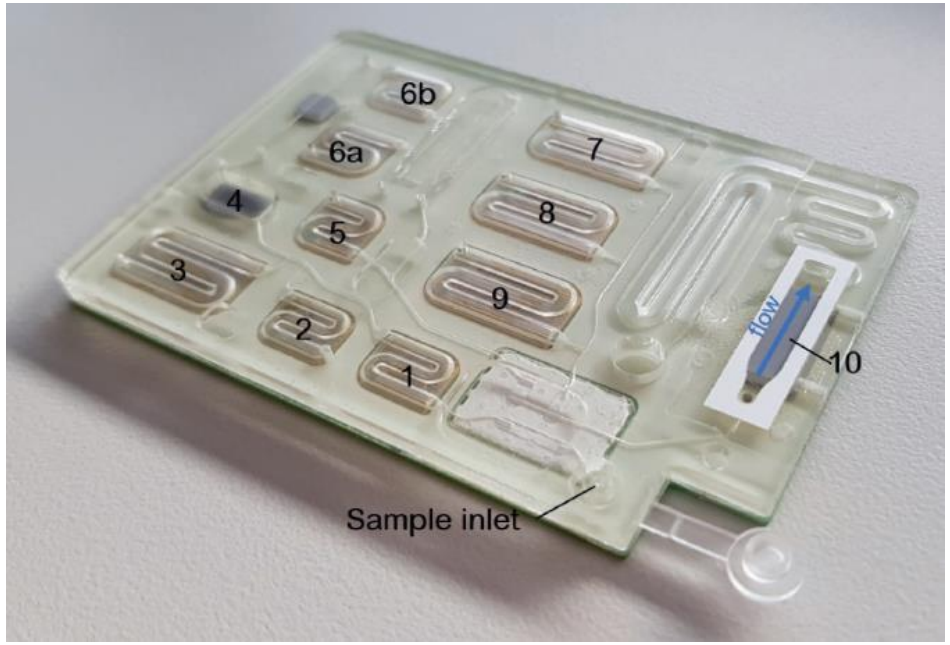
On-Chip
pumps + reagents



Real cartridge and instrument

Microfluidic Cartridge with
integrated pumps and heaters.
Functional elements are:

(1) Sample reservoir
(2) Amplification reagent reservoir
(3) Push buffer to empty (4)
(4) Amplification chamber
(5) Prehybridization buffer
(6a) Hybridization buffer
(6b) Reservoir for amplified sample
(7),(8),(9) Wash buffers
(10) Heatable sensor area with flow cell adhesive tape (white) for microarray foil attachement



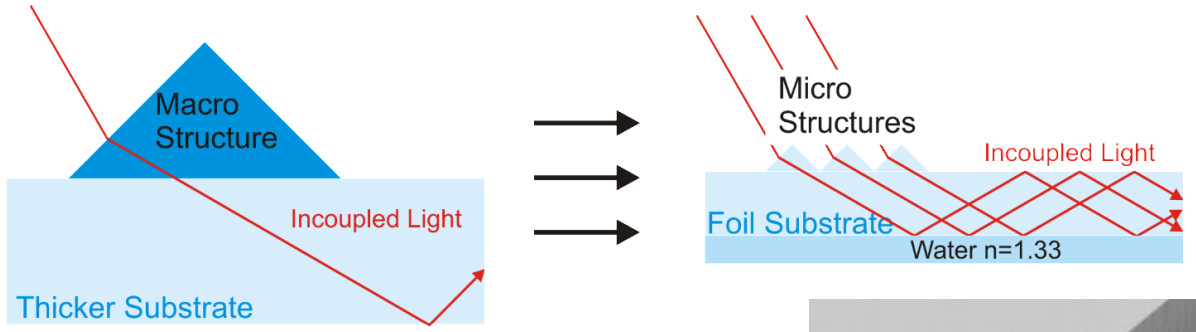
Microfluidic cartridge with pumps & heaters

Cartridge-control and Read-out Instrument Prototype

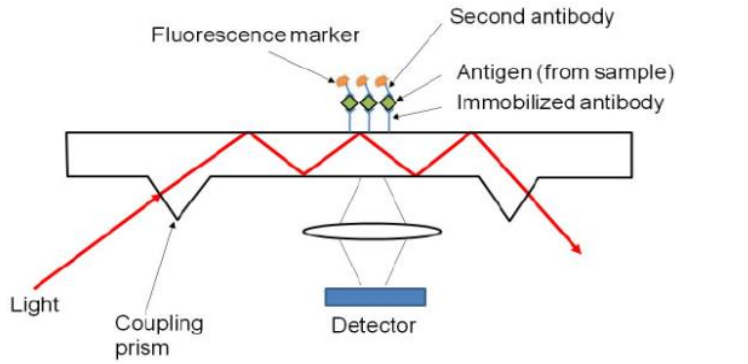
Note: here, a cartridge for amplification with RPA is shown, as cPCR-design is still confidential

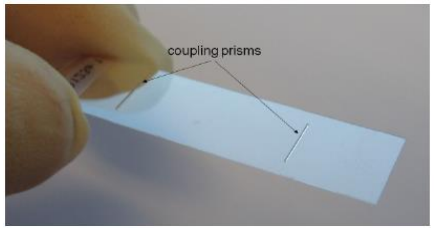
Sensor foil structuring

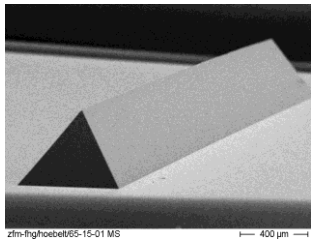
Replacement of existing macroscopic coupling prism by micro structures for light coupling on the sensor polymer foil.



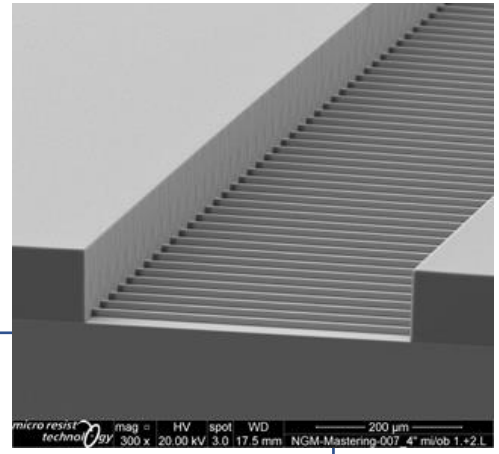
Existing system

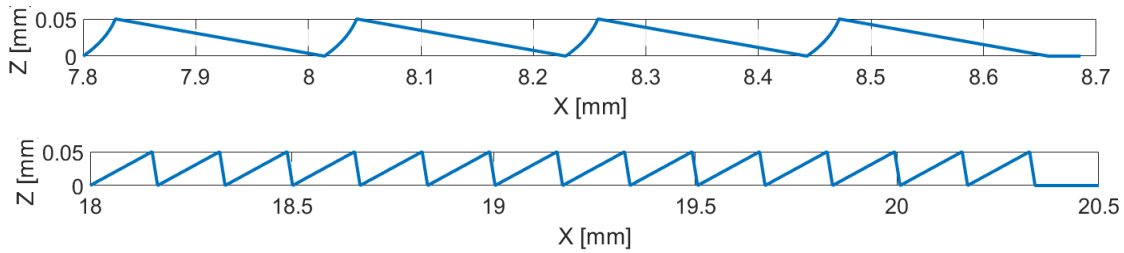






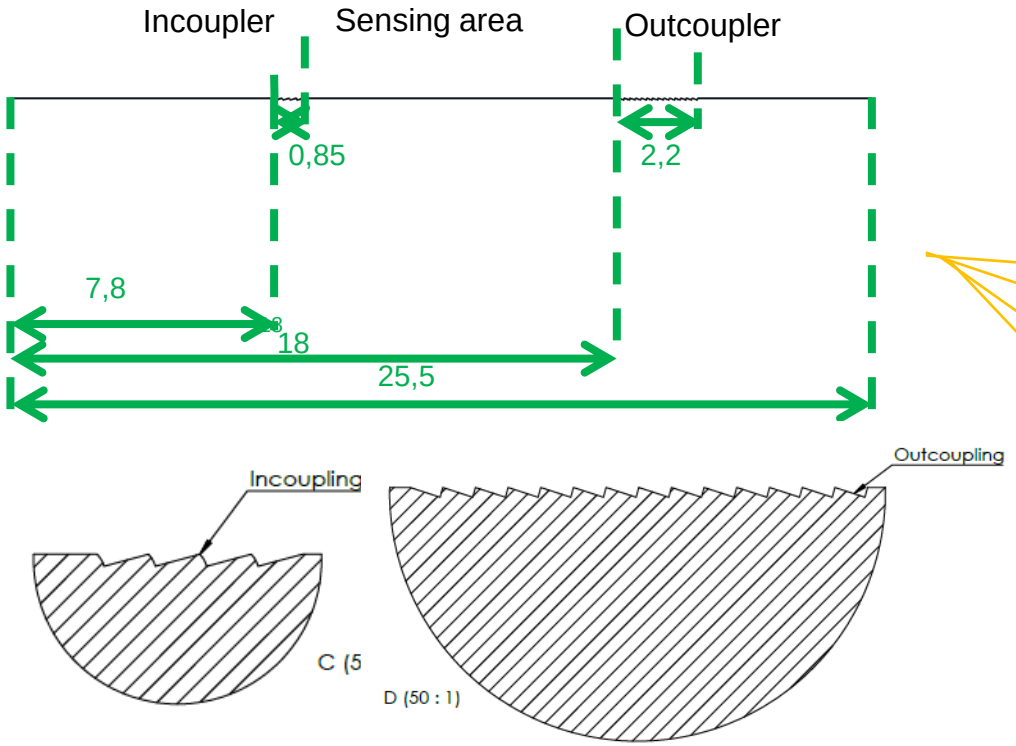
Optimized structures from simulation

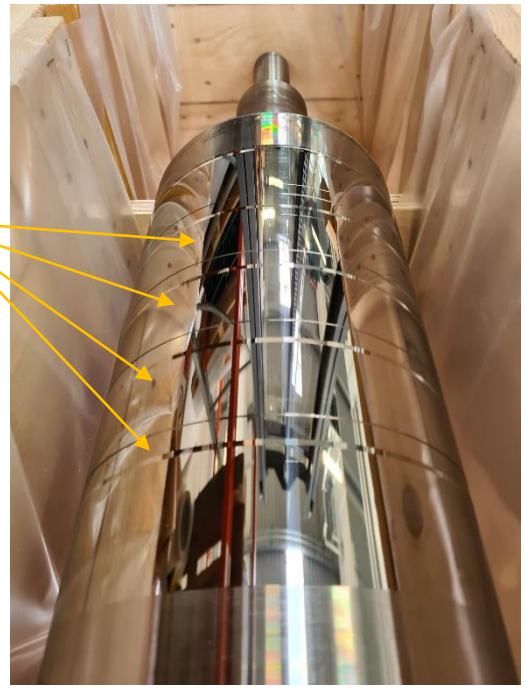




Replication of light in/out-coupling structures

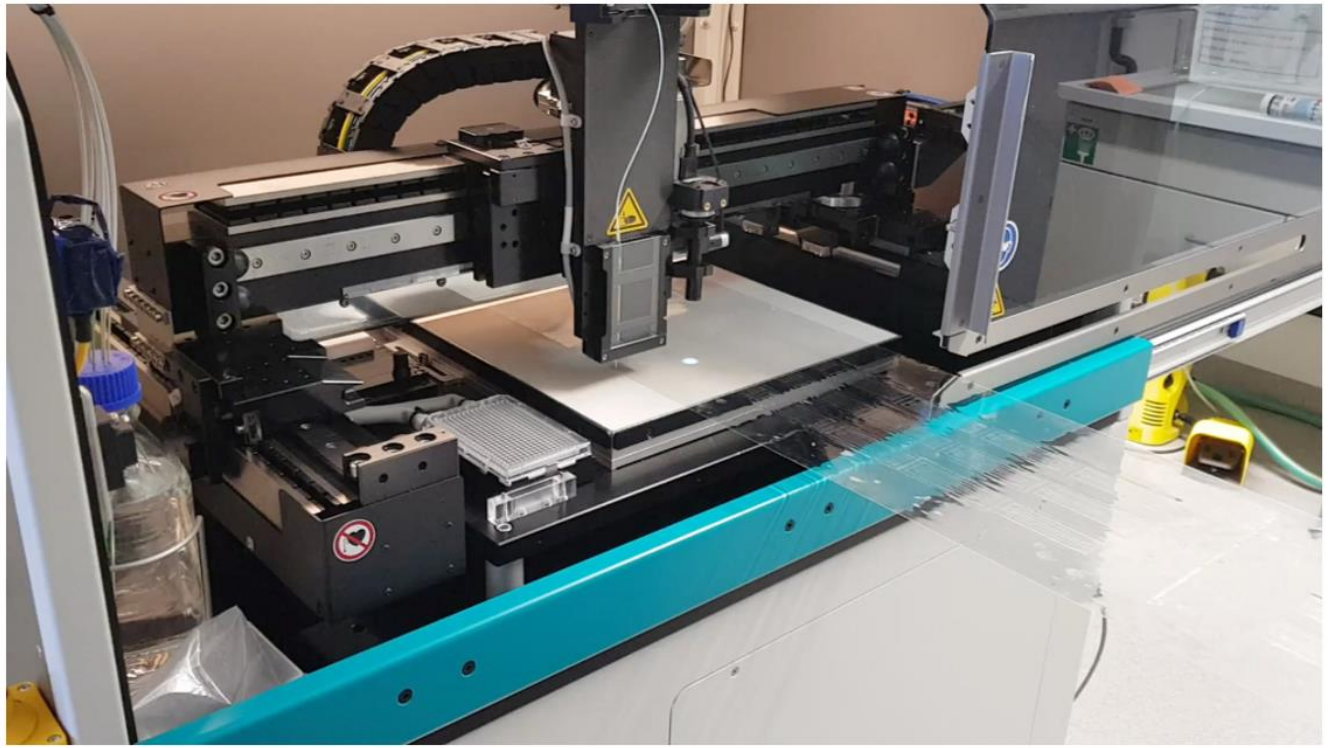
Engraved roller with the optimal in/out coupling structures





Layout of the Roller: Predefined distances between In-/Outcoupler (top); overview of the structures (below)

R2R Extrusion Coating:
Patterning of thermoplastic foils



Roll-to-Roll microarray spotting and sensor biofunctionalization

Positioning

	End-point PCR (for µArray)	qPCR	Point-of-Need Isothermal Amplification	Convective End-point PCR (for µArray)	Convective qPCR	Point-of-Need Convective End-point PCR (for µArray)
Speed (minutes, typ.)	**30-60** (w/o hyb.)	**20-40**	**10-30**	**10-30**	**10-20**	**10-30** (w/o hyb.)
Parallelization (reactions/instrument)	**++**	**++**	Limited, but possible	+(+)	+(+)	Limited, but possible
Ease of use	**-**	**-**	**++**	**-**	**-**	**++**
# bp	**up to >2000**	**up to >2000**	**typ. <500**	**up to >2000**	**up to >2000**	**up to >2000**
Multiplexing (amplification)	**++**	**+** (limited by # of dyes)	**--** (often only possible with multiple wells)	**++**	**+** (limited by # of dyes)	**++**
Targets per run	**up to 100** (multiplexing + microarray)	**<= 6**	**1-3 (?)**	**up to 100** (multiplexing + microarray)	**<= 6**	**up to 100** (multiplexing + microarray)
Assay translation from Lab-PCR	**++**	**++**	**-** (different primers, reagents, …)	**+**	**+**	**+**
Market readiness	**++**	**++**	**++**	**+**	**+**	**-**

SARS-CoV-2 gold standard

Majority of SARS-CoV-2 Point-of-Need molecular diagnostic solutions

This work

This work

Open Innovation Test Bed *NextGenMicrofluidics*

21 partners covering the **entire value chain from design to manufacturing** of microfluidic devices

Design & Simulation

BIONIC SURFACE TECHNOLOGIES

Materials

condensia Passion for chemistry

micro resist technology

Electronics manufacturer

Infineon

Bioprocess

NST WIRBELSCHICHTTROCKUNG

Process development

scienion ENABLING LIFE SCIENCE

INMOLD

temicon micronano solutions

RESCOLL Société de Recherche

Microfluidics development & manufacturing

bi.FLOW systems biofluidic integration

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

Sveučilište u Splitu

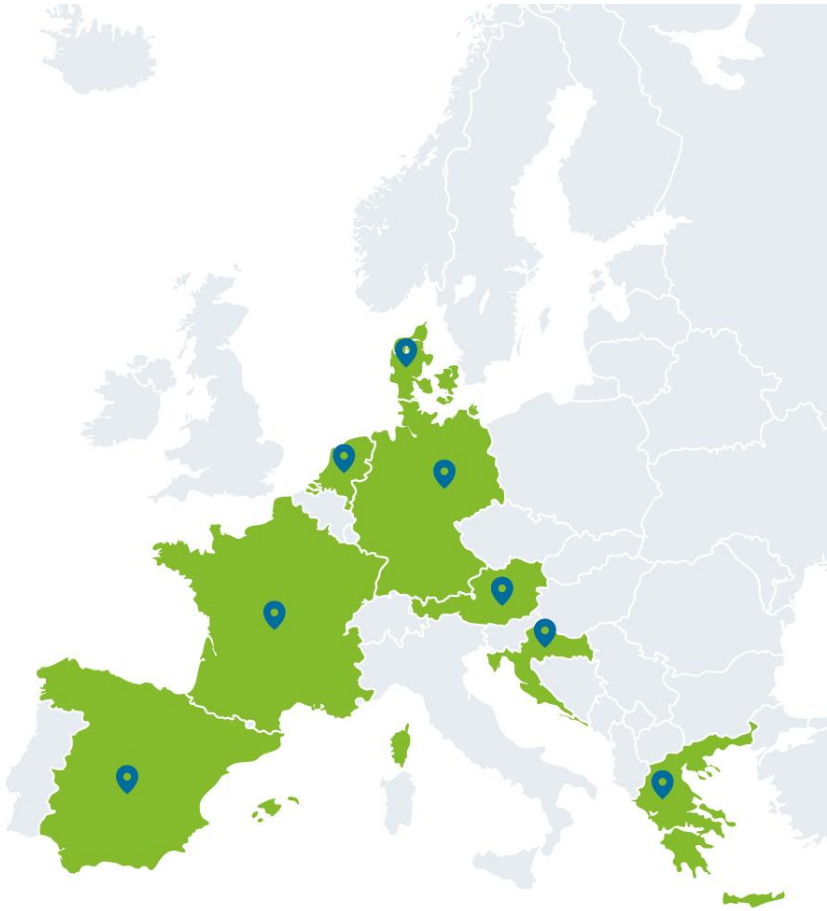
tecnalia Inspiring Business

TU Graz

BRFAA BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

BNN

JOANNEUM RESEARCH



Open Innovation Test Bed *NextGenMicrofluidics*

Open for broad range of applications

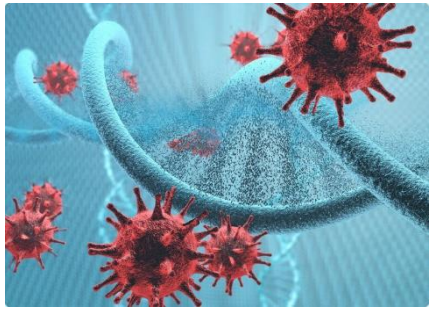
DEMO CASE 1
Biosensors for Food Safety Monitoring and Medical Diagnostics

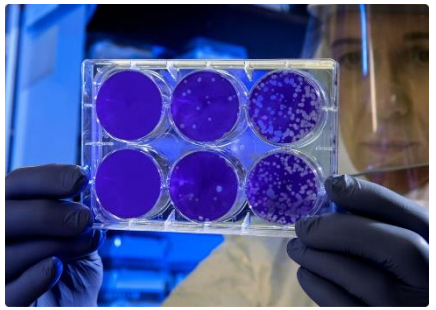
DEMO CASE 2
Sars-CoV-2 antibody detection

DEMO CASE 3
Smart Phone Enabled Home Diagnostics for Potassium in Blood

DEMO CASE 4
Cell Culture Devices for Pharmaceutical Testing

DEMO CASE 5
Sensors for Bio-Process Monitoring

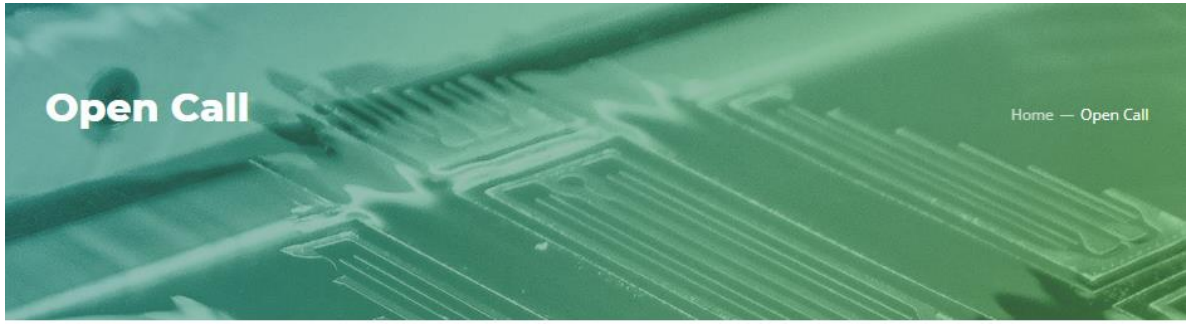




Single Entry Point *Microfluidic Innovation Hub*

NextGen Microfluidics

https://www.nextgenmicrofluidics.eu/microfluidics-innovation-hub/



GUIDELINE FOR APPLICANTS

Accelerate your Microfluidic Innovation

Scope of the NextGenMicrofluidics Open Call

The NextGenMicrofluidics Open Call provides a wide range of development support services in the field of Microfluidics to SMEs or Large Enterprises (LE). The goal is to accelerate the development and commercialization of innovative solutions enabled through foil based microfluidic chips (lab-on-a-foil systems) or microfluidic composite devices with foil based components.

✓ **Open call is now open**

OUR SERVICES

What We Offer

We develop and produce customized microfluidic lab-on-a-foil systems.

NextGenMicrofluidics will implement technologies and services that allow maximal adaptability to a vast majority of potential customer inquiries in microfluidics.

Simulation Based Design

We design the optimal structures for your microfluidic system.

Assay Development

We develop biochemical and molecular assays for your specific requirements.

(Bio)Functionalization

We deposit and immobilize your (bio)functional material in your lab-on-a-foil.

Material Development

We develop resins, functional inks or selective membranes for your microfluidic system.

Mastering of Structures

We prototype your required 3D-structures and complex nanopatterns on a large scale.

Chip Production

We offer unique roll-to-roll based production processes for continuous high throughput manufacturing of your microfluidic consumables.

THANK YOU

https://www.nextgenmicrofluidics.eu/

This project has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.