Microfluidics InnovationHub

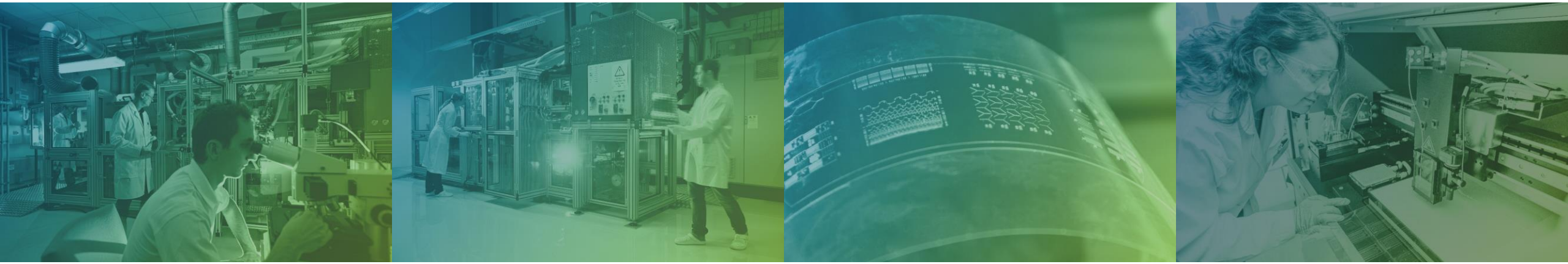
We get Microfluidics rolling

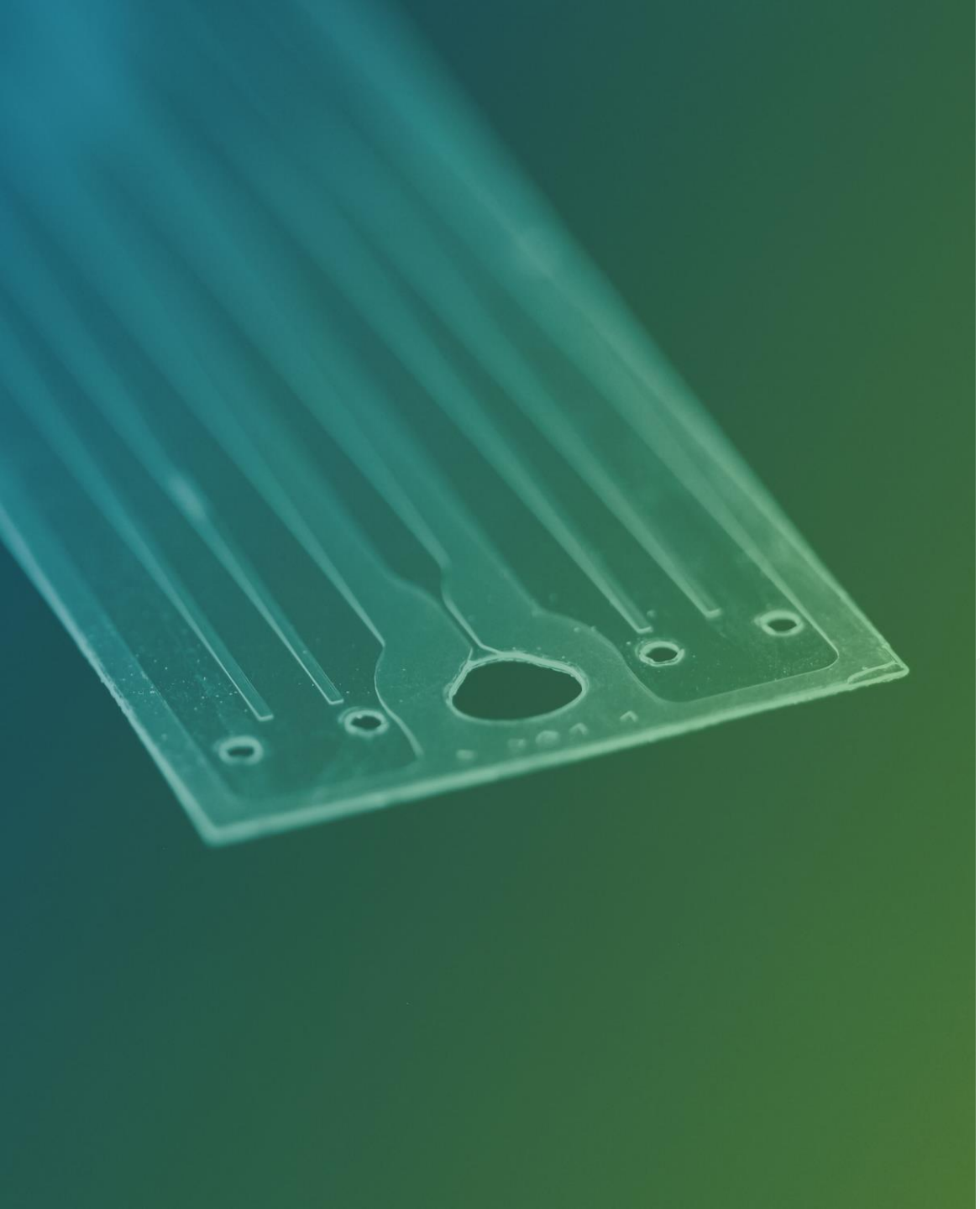
The Microfluidics Innovation Hub (MIH) is the single-entry point of the European project NextGenMicrofluidcs (NGM) which has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.

Microfluidics InnovationHub

NextGenMicrofluidics Demo Case 5

Milestone Report





NGM DEMO CASE 5

- **Application:**

Enzyme activity monitoring from enzyme extracts derived from large scale bioprocess fermentation

- **Measurement parameters:**

One-step colorimetric assays for measuring the enzyme activity

- **Measurement principle:**
  - Immobilization of enzyme substrate on microfluidic chip chamber
  - Colour change resulting from enzymatic catalysis of specific enzyme substrate
  - Quantification of enzymatic reaction products via spectroscopic absorbance measurements

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Point-of-Need Lab-on-a-Chip Industrial Enzyme Detection

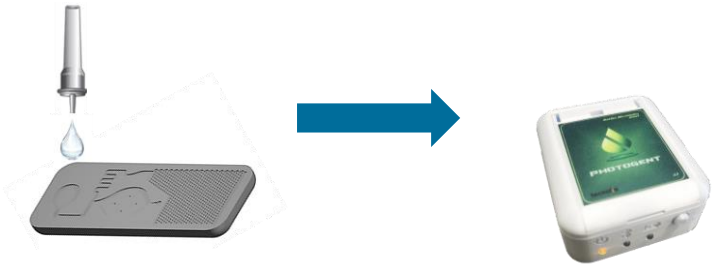
Use Case:

- Monitoring of enzyme production during large scale fermentation
- Quality control of enzyme-based products
- R&D – development of new enzymes and production vectors, enzyme inhibitors, reactions conditions etc.

Benefits:

- **Laboratory quality results**
  - Performance comparable to laboratory methods
    - Quantification of enzyme activity (sensitivity?)
    - Real-time rate determination
- **Rapid Measurements** (>10 mins following sample prep)
- **Low cost**
- **On-site measurements**
  - Unskilled operation
  - Low footprint
  - On-chip reagents
  - Storable up to 6 months
  - Digital integration
- **Flexible system**
  - Multiple enzyme detection.
  - Applicable to different raw products and markets

Industrial fermentation for enzyme production and stabilization



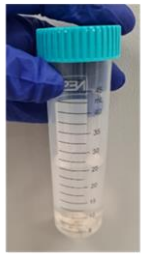
Microfluidic chip for measuring enzyme activity with colorimetric read-out device

Portable read-out device

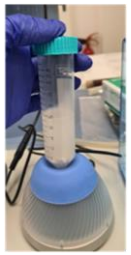
User Operation

1. Sample preparation

Collect and measure raw sample



Add extraction solvent and microbeads



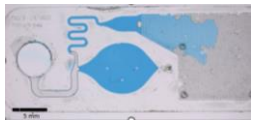
Vortex

Extract liquid phase using syringe filter

2. Sample loading

Transfer sample to dropper vial containing reaction buffer

Transfer drop onto microfluidic chip inlet



Sample automatically fills reaction chamber

3. Read-out

Small footprint colorimetric absorbance (405 and 570 nm) read-out device with integrated heating

4. Data collection

Real time measurements of enzyme activity (kU/mg) are obtained as change in absorbance over time and transmitted via bluetooth to a smartphone.

Android app

Work Flow

Assay Development

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Microfluidic Chip Design

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BIONIC SURFACE TECHNOLOGIES

Extrusion Coating Roll-to-roll manufacture

INMOLD

Reagent microspotting

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Backend processes

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Portable read-out device

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Device Testing and Validation

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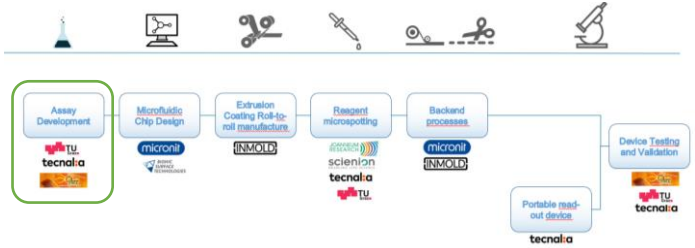
Molecular Workflow



Enzymatic reaction → colour generation

On-chip reagents are catalyzed in the presence of the target enzyme into reaction products with absorbance at specific wavelengths that can be detected by absorbance spectroscopy.

Requirements

- Enzymatic assay compatible with lab-on-chip development by R2R extrusion coating process: no filtration or precipitation steps
- One-step assay to simplify the process and take advantage of synergies in chip design

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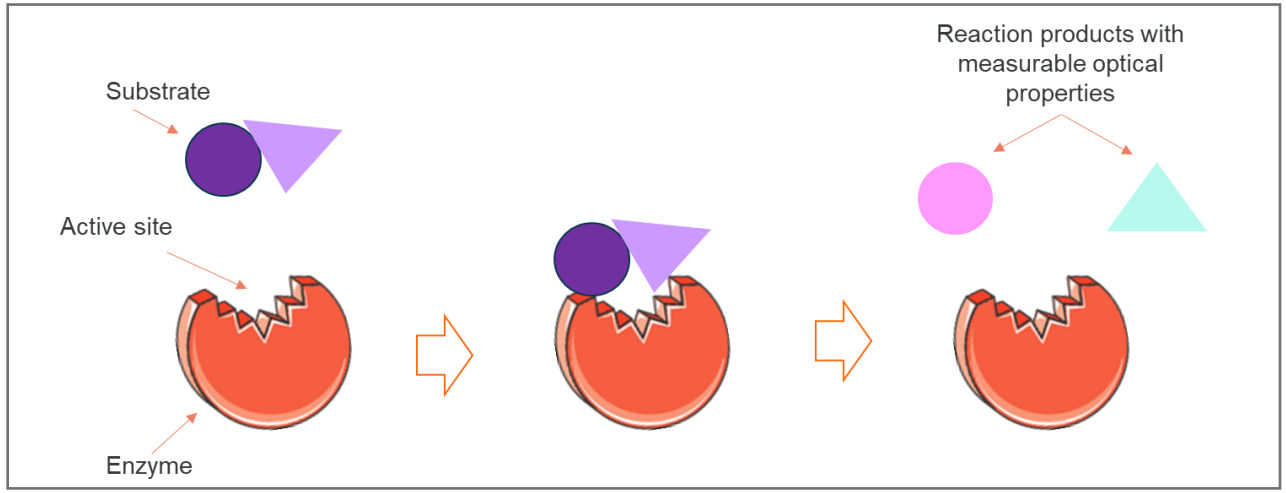
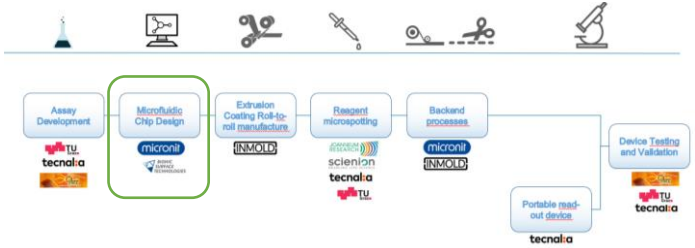


Enzyme substrates bound to the microfluidic chip surface are catalysed into enzymatic reaction products with measurable optical properties in the presence of a sample containing the right enzyme.

Operating Principle – Microfluidic Chip



The user adds a drop of the sample containing the enzyme in the inlet and the chip is self-filled by capillary action. The sample is delivered to the reaction chamber where the enzyme substrate is stored. The flow continues towards the capillary pump structure, removing excess sample and isolating sample in the detection chamber.

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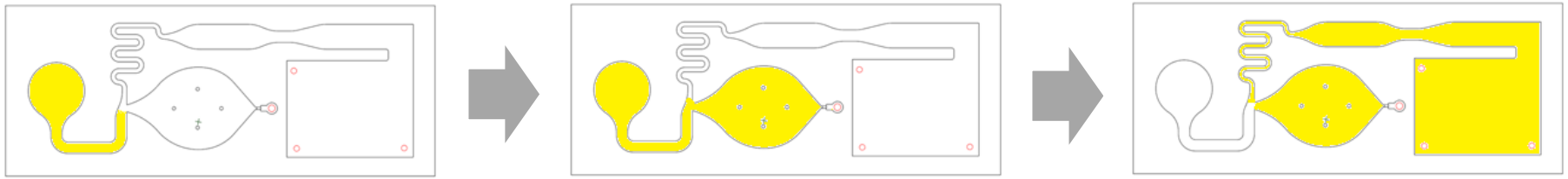


User adds a drop of sample (dried reagents integrated on the chip)

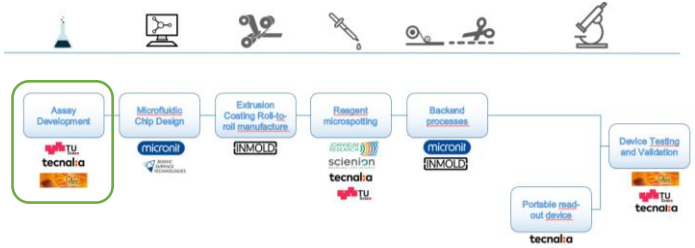
Self-filling by capillary action

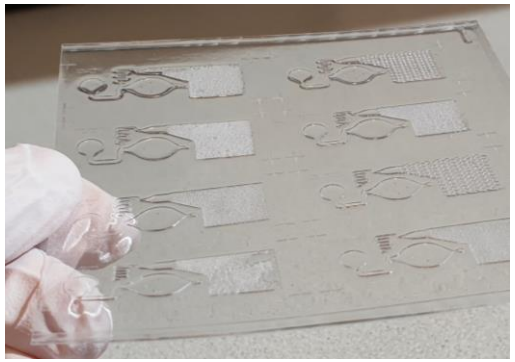
The hydrophilic pump removes the liquid excess isolating the reaction chamber.

Operational Features

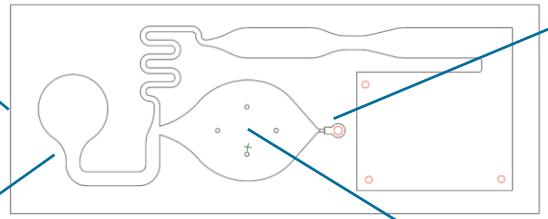
- **Hassle free inlet:** Microfluidic design enables hassle-free and reproducible, bubble-free filling of the reaction chamber via contacting a drop of fluid with the inlet.
- **Volume metering:** The reaction chamber is filled with a fixed amount of fluid and isolated from excess fluid, which is stored on-chip in the capillary pump structures for easy disposable.
- **Quantification:** The combination of a fixed amount of sample volume and dried reagents enables absolute quantification of enzyme activity and/or concentration.
- **Storage:** Fridge-stable for up to 6 months**.** Low footprint allows for easy storage (chip dimensions 1 cm x 2 cm x 0.1 cm).

Microfluidic Chip Design Overview

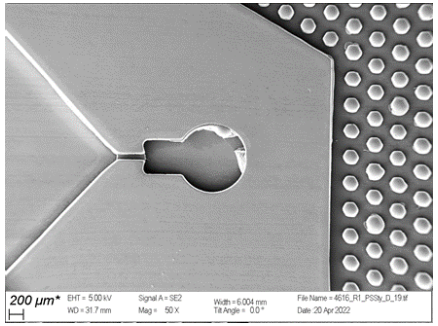




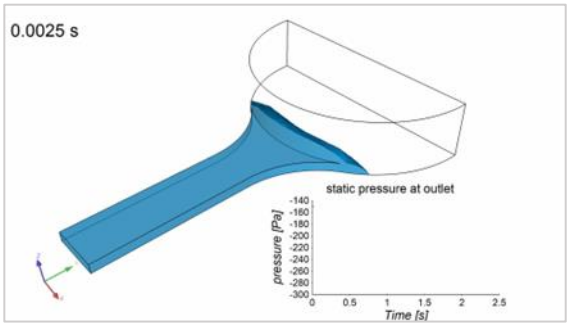
Roll-to-roll manufacture on polymer for high-volume, low cost manufacturing



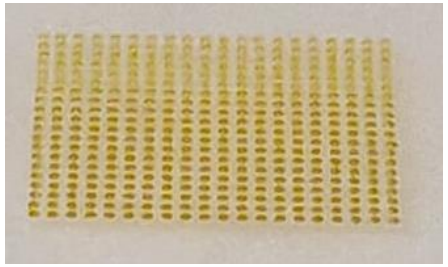
Microfluidic chip design



Microfluidic stop valve and vent structure for efficient filling of the measurement chamber

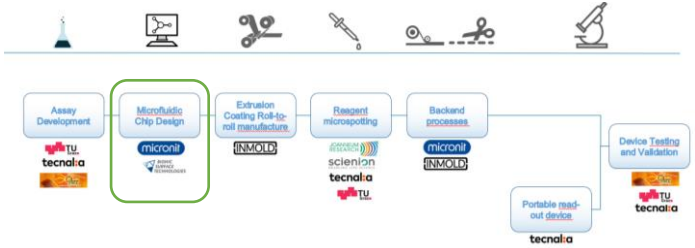


Inlet and capillary pump design informed by Computational Fluid Dynamics (CFD) for rapid and reproducible fluid flow



Microspotting of enzyme substrate reagents onto measurement area surface ensures homogenous enzyme-substrate reactions and controlled reagent concentration, enabling rapid and absolute quantitative measurements

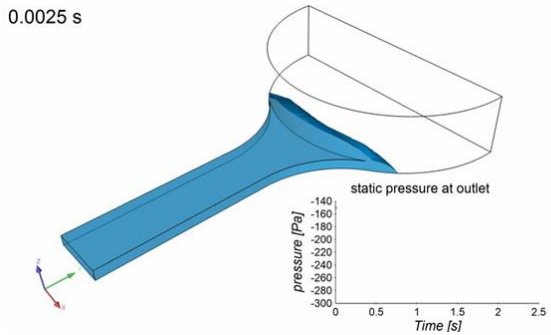
Computational Fluid Dynamics (CFD)



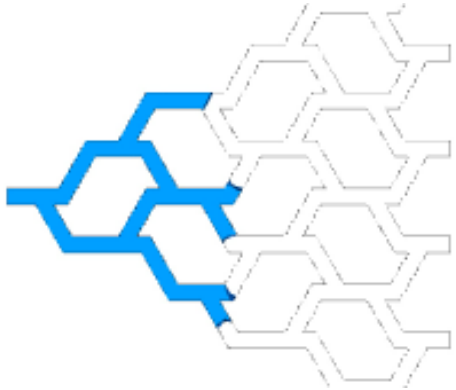
BIONIC SURFACE TECHNOLOGIES

Computation Fluid Dynamics (CFD) simulations are employed to aid the design of the microfluidic chip.

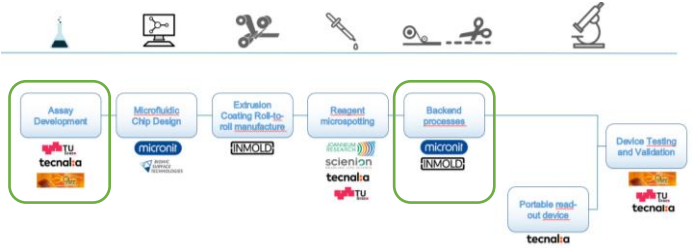
Inlet Design Optimization
Simulation-assisted design of inlet ensures reliable and user-friendly sample delivery and informs the required pressure to break the meniscus. This provides a starting point towards the design of the capillary pump.



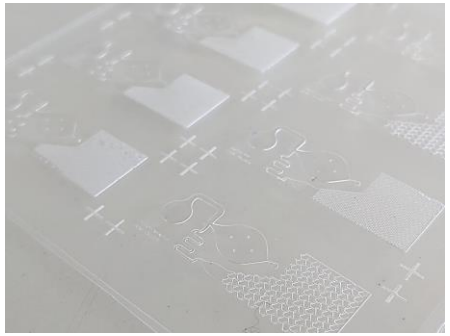
Capillary Pump Design
Capillary pump geometries are designed in order to ensure rapid and reliable flow through the chip ensuring the measurement chamber is always filled.



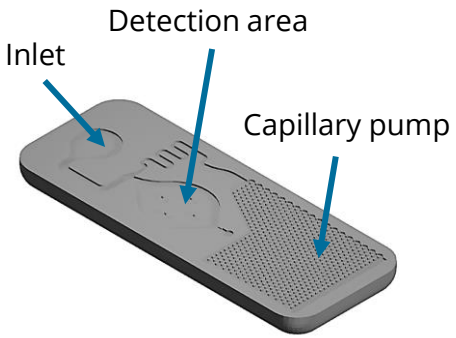
Microfluidic Chip Design and Assembly Process



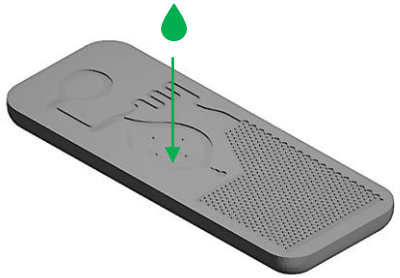
1. Extrusion Coating Roll-to-Roll (EC R2R) Imprinting of microfluidic structures



2. Singulation of chip unit



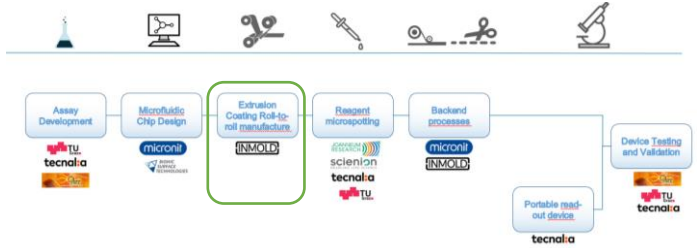
3. Application of enzyme substrate reagents



4. Lamination with pre-cut adhesive tape

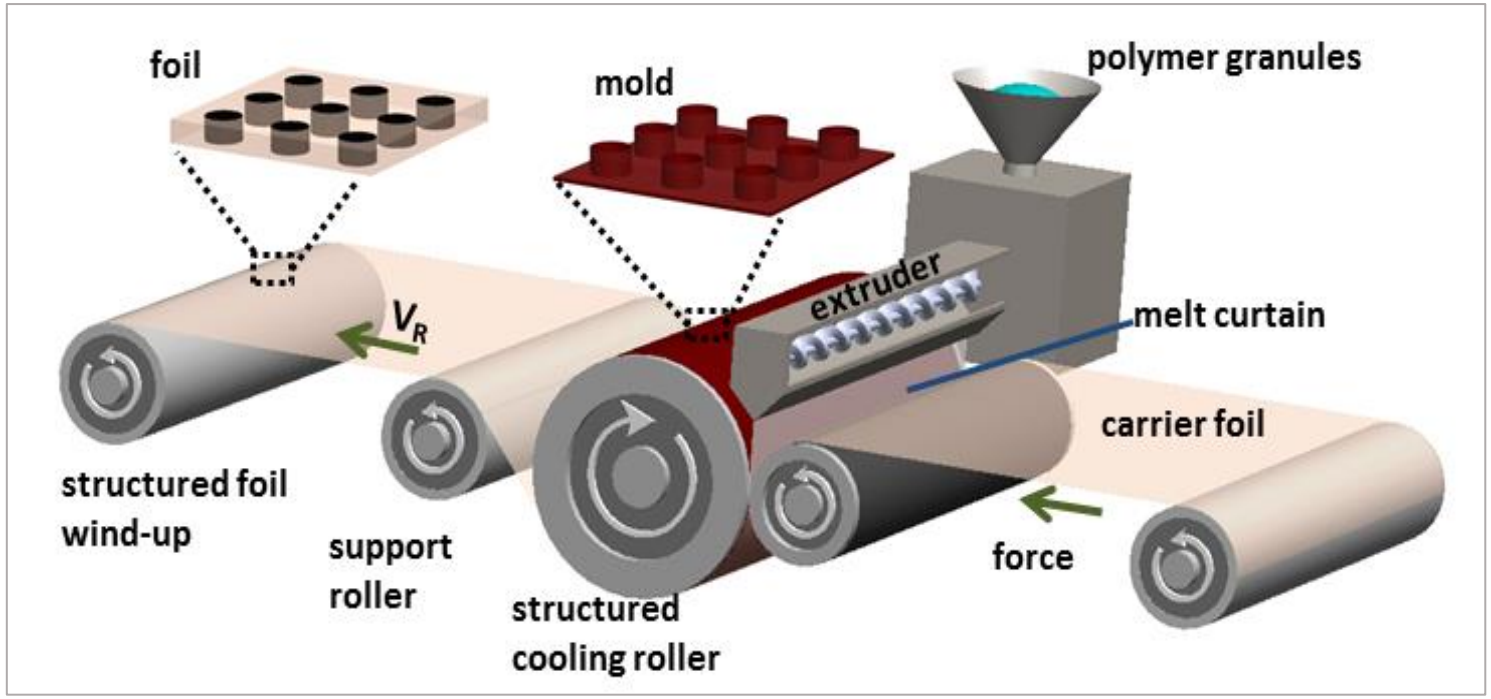
ready to use with sample!

EC R2R Imprinting



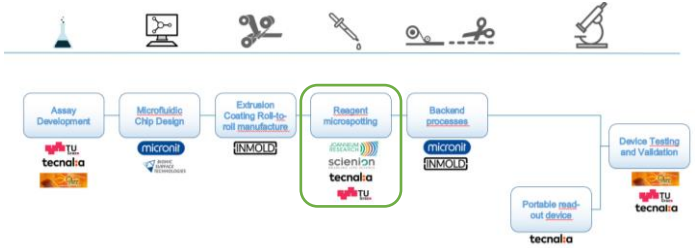
INMOLD

EC R2R manufacture of the microfluidic structures ensured high manufacture fidelity as well as low cost and high volume production

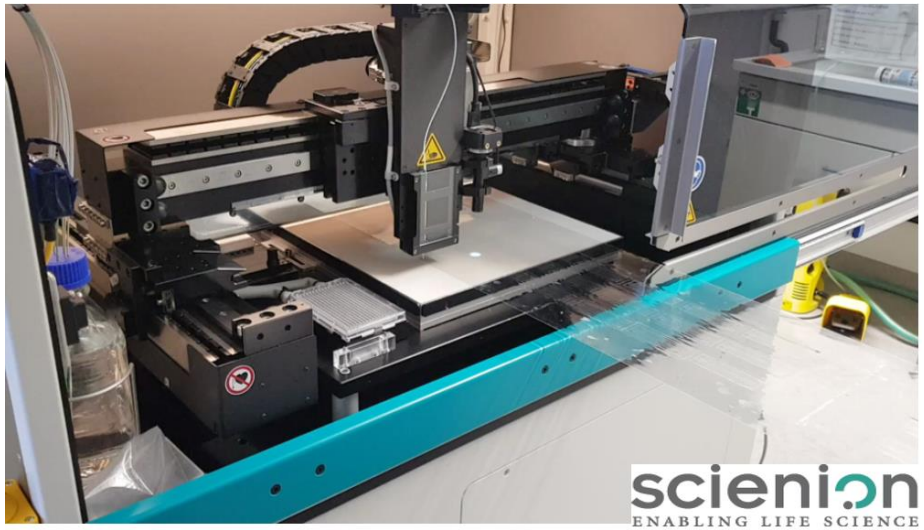


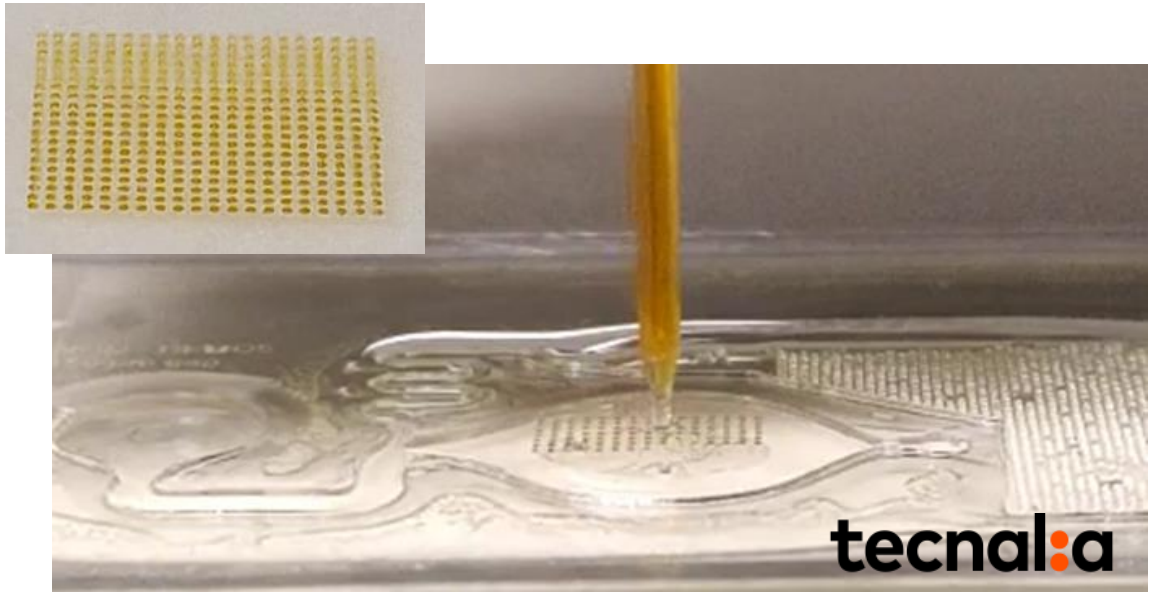
Murthy & Matschuk et al., (2016) Advanced Engineering Materials, 18 , 484-489

Spotting of Enzyme Reagents



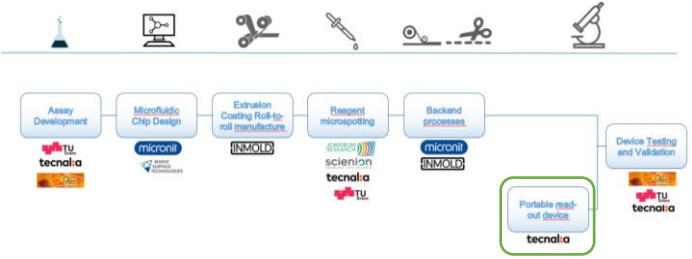
Piezoelectric deposition using a microspotter is employed to deposit enzyme substrate reagents onto the measurement chamber of the microfluidic chip. Microspotting ensures low chip-to-chip variability of reagent concentration and reaction kinetics.





20x20 spots; 5 drops/spot

Portable Read-Out Device



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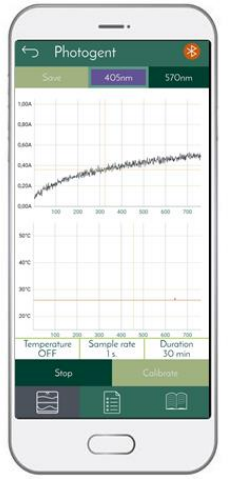
A portable, stand-alone read-out device is in development to combine spectrophotometry for absorbance read-out at specific wavelengths and also to achieve localized heating of the reaction chamber.

- ✓ Detection chamber optimized to the chip design
- ✓ Independently controlled 405 nm (UV) and 570nm (green) narrow bandwidth photodiodes
- ✓ Photodetector with transimpedance amplifier
- ✓ Algorithm for estimation of absorbance A = log(I0/I)
- ✓ Resistive heating elements
- ✓ Bluetooth 5.0 low energy
- ✓ Indication LEDs (yellow – status, blue –connection, red - charging) and battery charging feedback

Milestone Report

- ✓ Microfluidic device design and manufacture complete
- ✓ First prototypes for the read-out system produced
- ❑ First measurements using enzyme samples currently in progress
- ❑ Android app for device control and data logging in development
- ❑ System validation in industrial setting pending



Device control and read-out app currently in development

Validation of entire system will be performed at the enzyme production facilities of Naturstoff-Technik GmbH

…Stay posted for updates in Q3 2023!

THANK YOU!

We look forward to working with you

Contact Us

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