MICRO- NANO- DEVICES FOR BIO

How to develop a Lab on a chip and a biosensor

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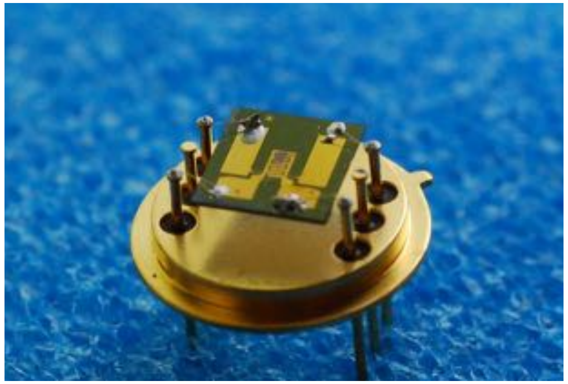
Chi-Lab *- Dipartimento di Scienza Applicata e Tecnologia*– **Politecnico Di Torino**

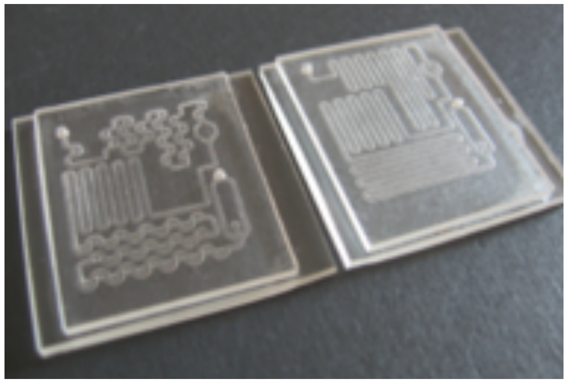
It-fab Italian Network for Micro and Nano Fabrication

Nano Innovation 2020 Rome, 15-18 September Conference & Exhibition

XLAB

imem





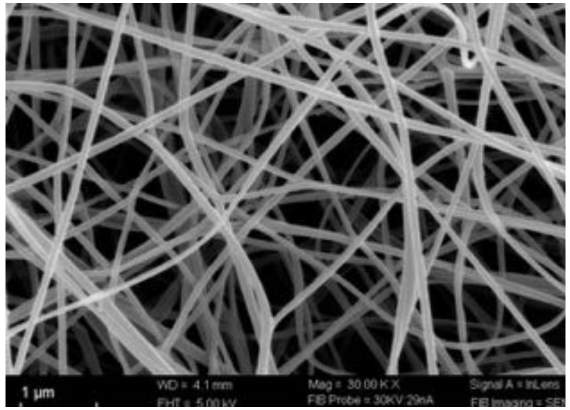
http://www.polito.it/micronanotech

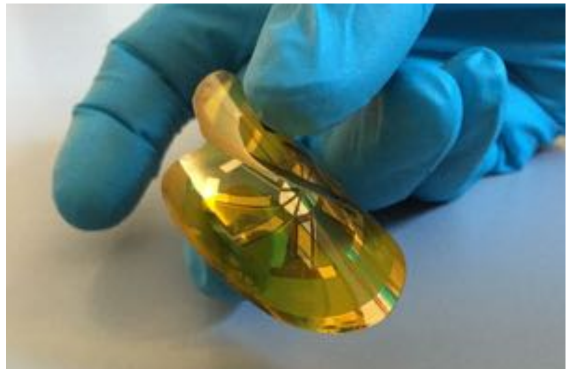
Mission

- Fundamental research on materials and processes for micro- and nano-technologies
- Design and fabrication of MEMS and nanostructures
- Technological transfer
- Education

Staff

- 7 Professors
- 5 Permanent Researchers
- 16 Fellowships / Post Doc
- 8 PhD students
- 2 Technicians
- 1 Administratives





Outlook

- Motivations
- Microfluidics
- Biosensing
- Development tools & Facilities
- A BAW microfluidic chip
- A nano EGO-FET biosensor

MOTIVATIONS

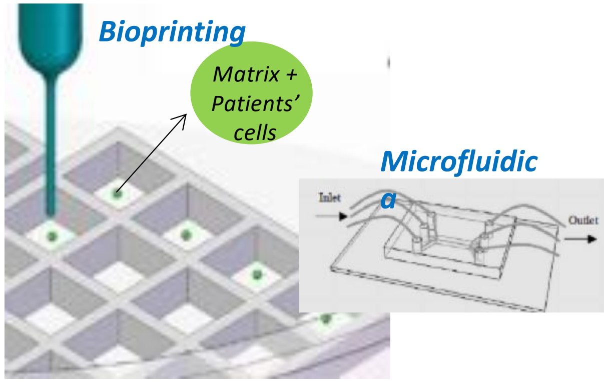
Oncologic diseases

Neurological disorders

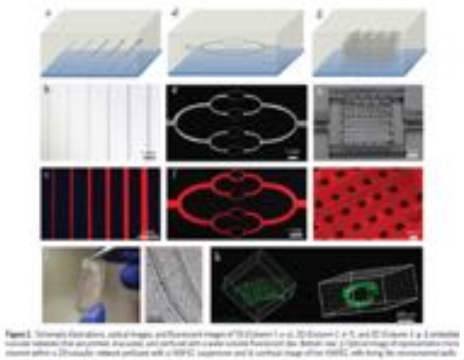
Regenerative medicine

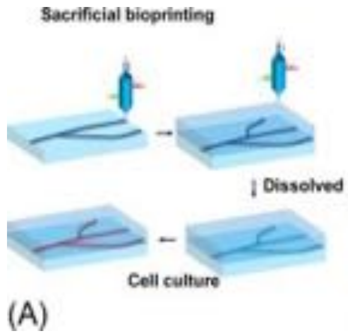
3D scaffold for Organ/tissue on chip

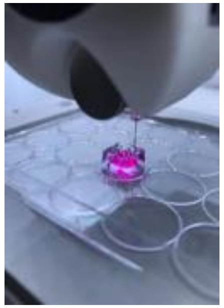
3D cells cultures



Scaffold, Tissues & Organs in silico



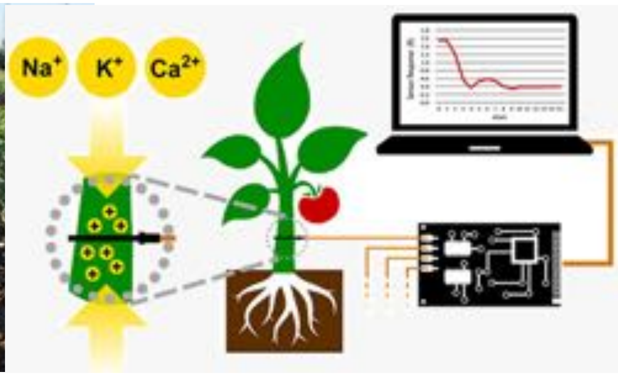




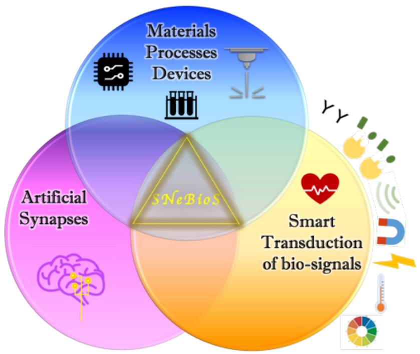
Motivations: health / food / environmental monitoring

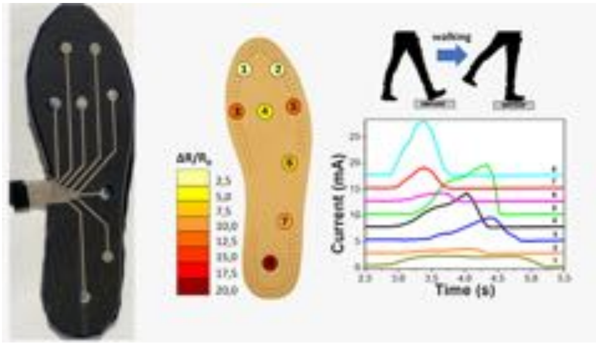
FOOD DRUG FREE

Food monitoring for cotaminants: antibiotics, toxins, chemicals…



Precise Agriculture
Water control
Air control for pollutant
…





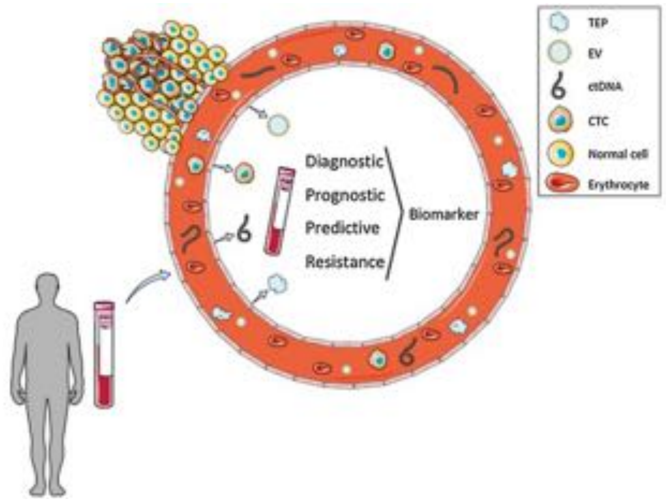
Chronic disease
Nanomedicine
drug delivery
Sport
…

Tool for diagnosis, prognosis, prediction of responce to therapy

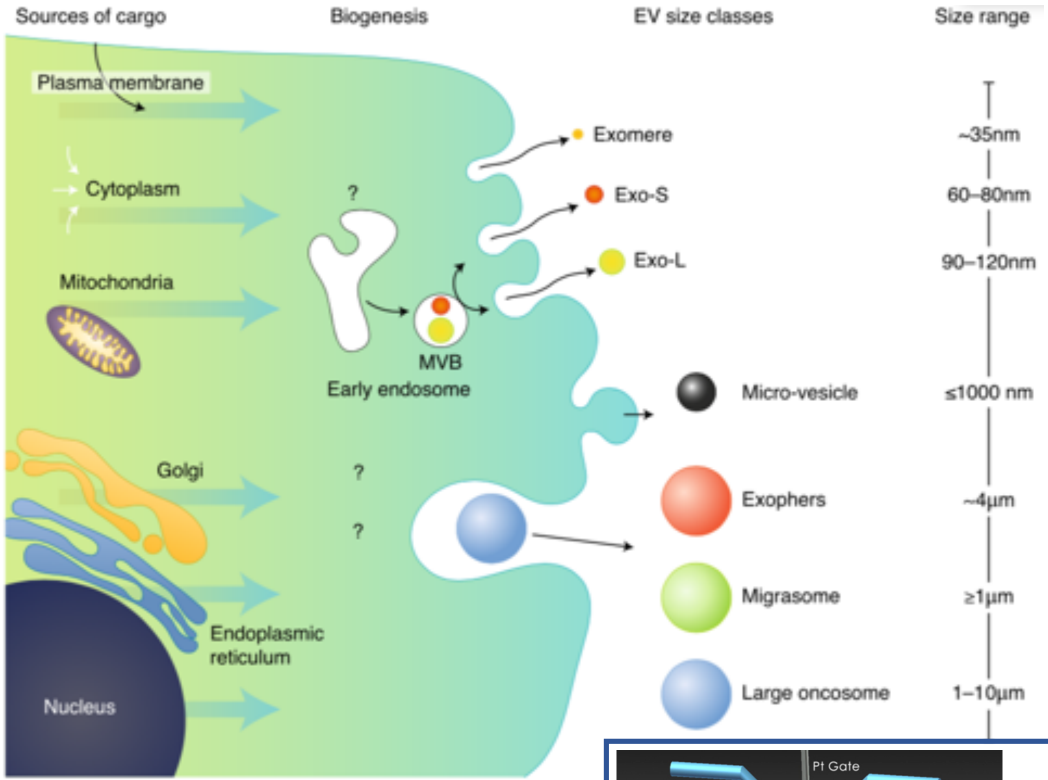
- TEPs tumor encapsulated platelets
- ctDNA circulating tumor DNA
- CTCs circulating tumor cells
- EVs extracellular vesicles

Advantages
Repeated sampling
Reflectation of the complete molecular status of disease

Bracht et al.; Current Oncology Reports (2018)
Paper on NON-SMALL CELL LUNG CANCER
TEPs and ctDNA have potential in disease monitoring and can indicate acquired resistance much earlier than radiography disease progression



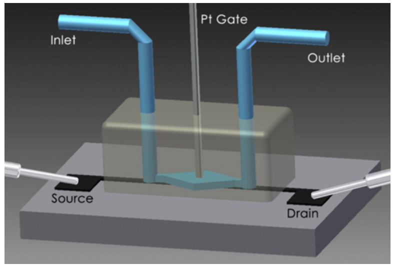
Motivations: Liquid Biopsy approach



- Exosomes are heterogeneous, nanoscale vesicles that mediate **cellular communication**
- EV are **promoters of cancer progression**: 1. transfer of active biomolecules to the tumour environment; 2. functional role of their cargo in sorting disease progression; 3. inhibition of EV secretion or biogenesis correlated with the drug resistance development
- **EV heterogeneity** is not only defined by **variation in size** but also by **variation in cargo between and within each size class**
- Exomeres and exosomes have different biogenesis, different cargo and different biodistribution
- Whereas the **biogenesis of exosomes is relatively well defined, biogenesis of larger vesicles and exomeres is currently unknown**

Di Vizio Nat. Cell. Biol. 2018

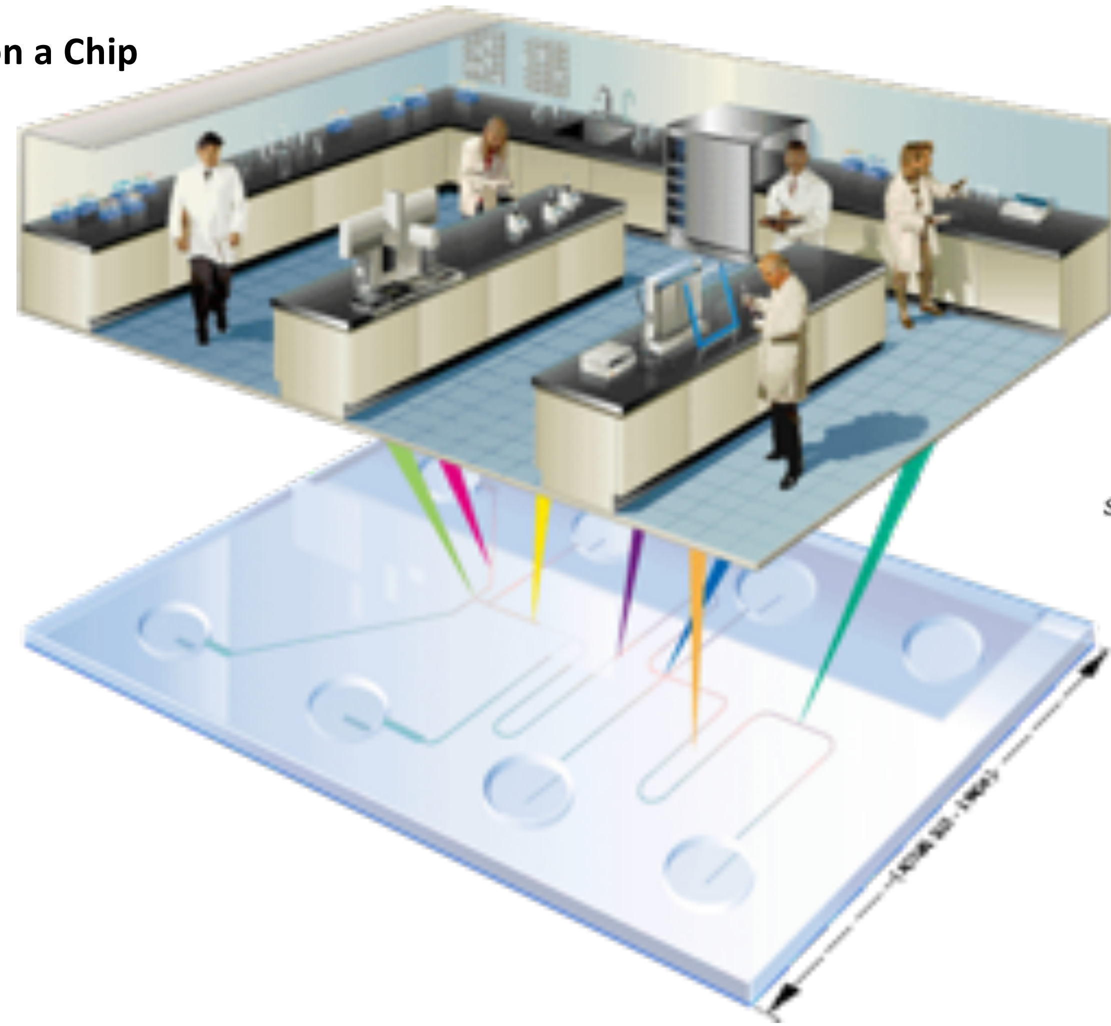
Biomarkers detection on fluidic integrated biosensors

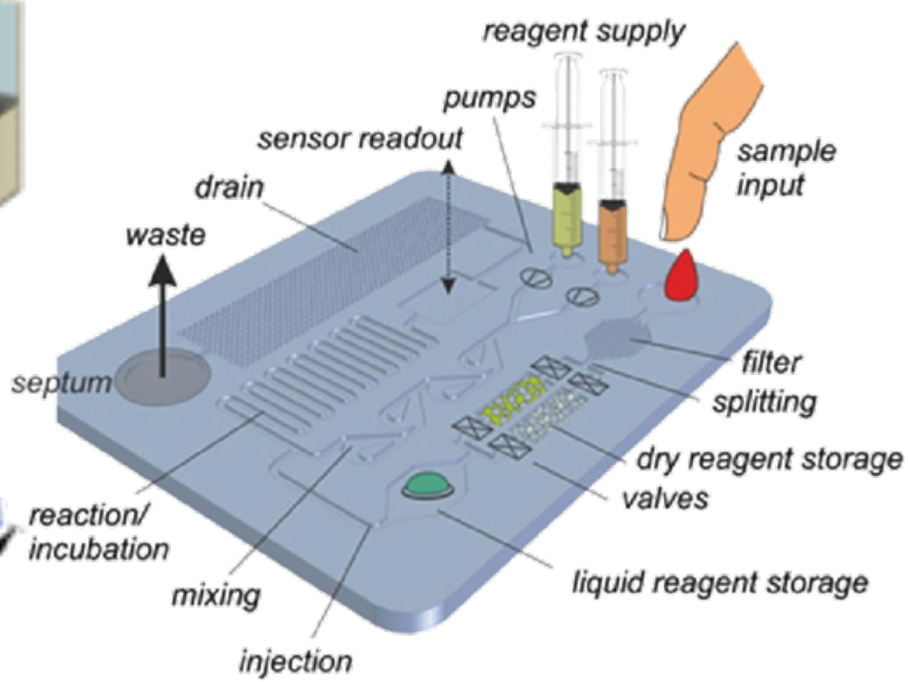


- Tarabella, Giuseppe, Balducci, Anna Giulia, Coppedè, Nicola, Marasso, Simone, D'Angelo, Pasquale, Barbieri, Stefano, Cocuzza, Matteo, Colombo, Paolo, Sonvico, Fabio, Mosca, Roberto, Iannotta, Salvatore. Liposome sensing and monitoring by organic electrochemical transistors integrated in microfluidics. Biochim. Biophys. Acta 2013, 1830, 4374–80.

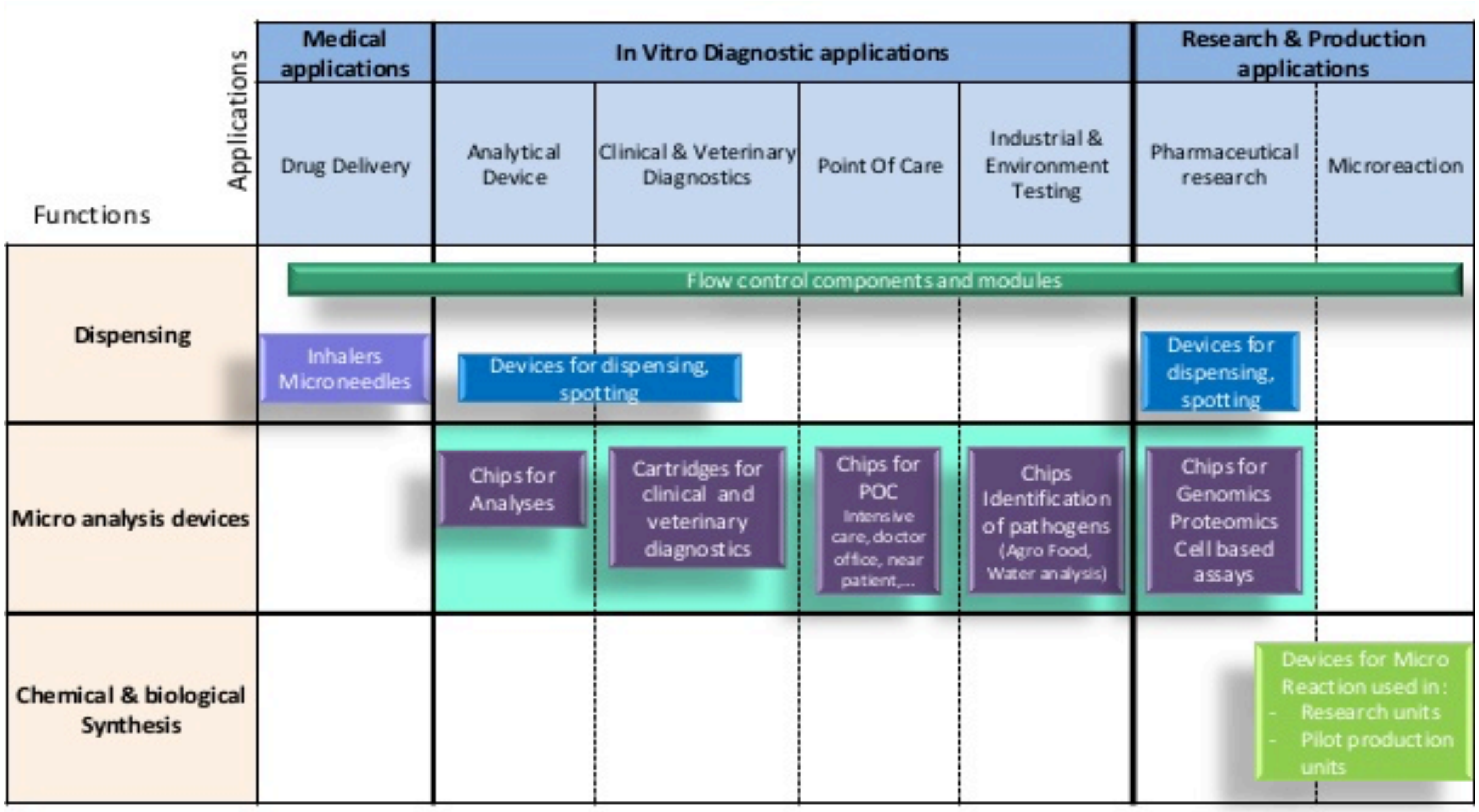
MICROFLUIDICS

Lab on a Chip

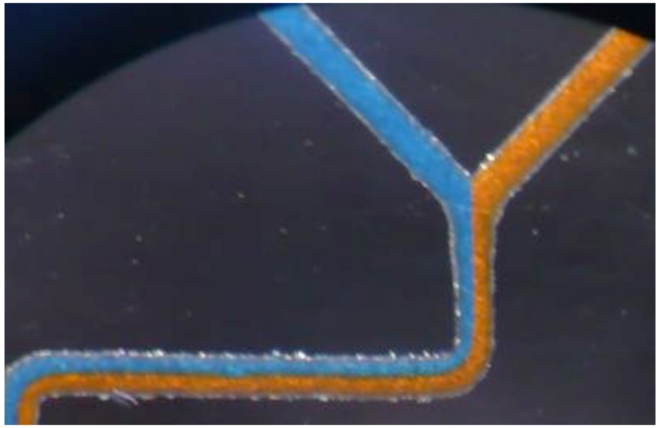


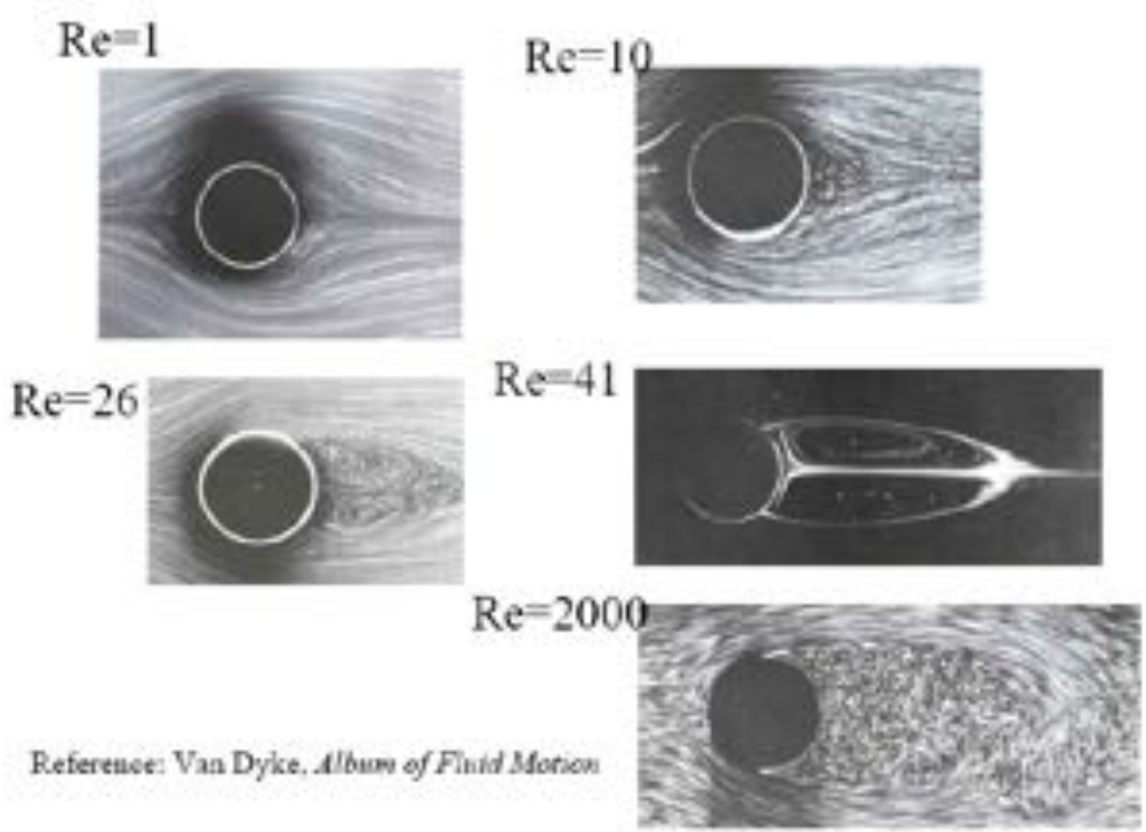

reagent supply
pumps
sensor readout
drain
waste
sample input
septum
filter
splitting
dry reagent storage
valves
reaction/ incubation
mixing
injection
liquid reagent storage

Microfluidics



- **surface forces are dominant;**
- **diffusion driven system;**
- **the flow is in laminar regime;**
- **viscosity of fluids are important.**





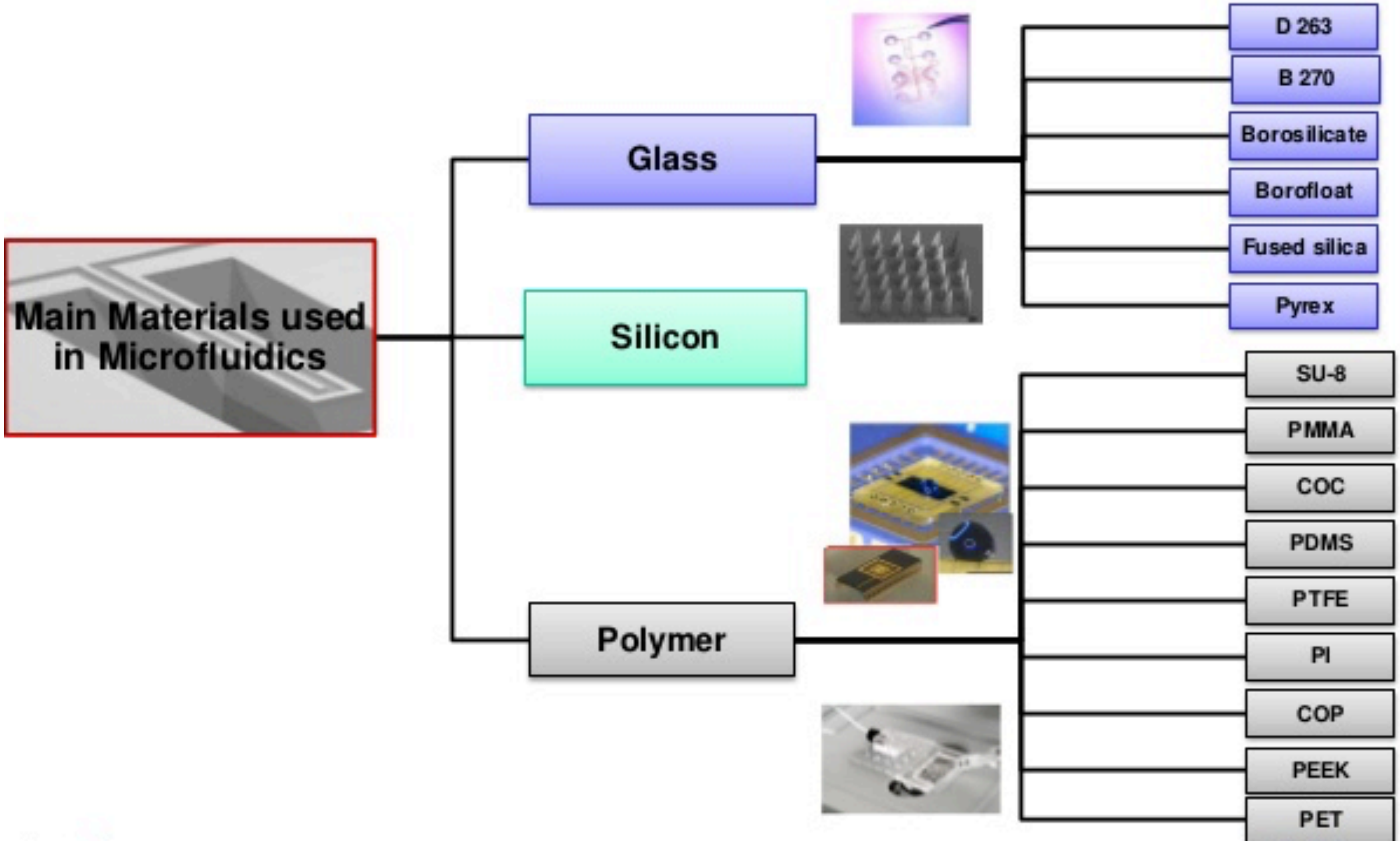
Materials:

Silicon micromachining

- Integrated circuits → Transport electron
- Microfluidics → Transport molecules and fluids

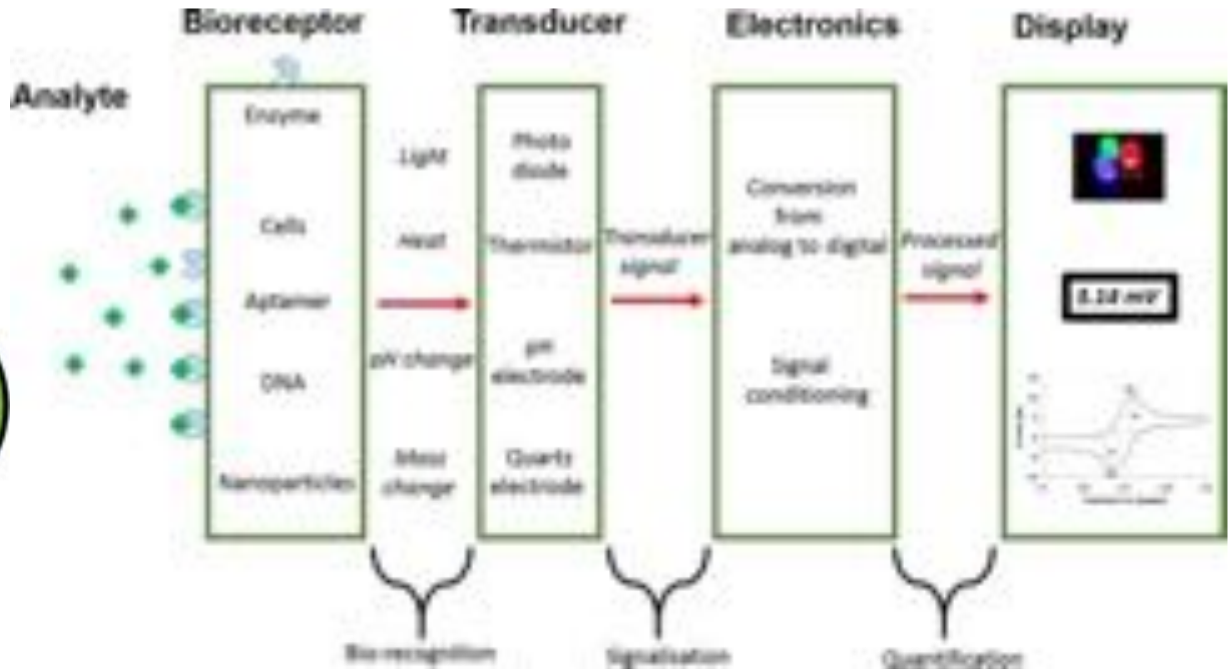
Plastic micromachining

- Stamp and mold
- Microcutting
- Laser machining
- Microelectro discharge machining
- Laminting



BIOSENSING

Biosensors and Biosensing



- **Selectivity**
- **Reproducibility**
- **Stability**
- **Linearity**

Essays Biochem. 2016 Jun 30; 60(1): 1–8.

Biosensing basic approach

Target definition

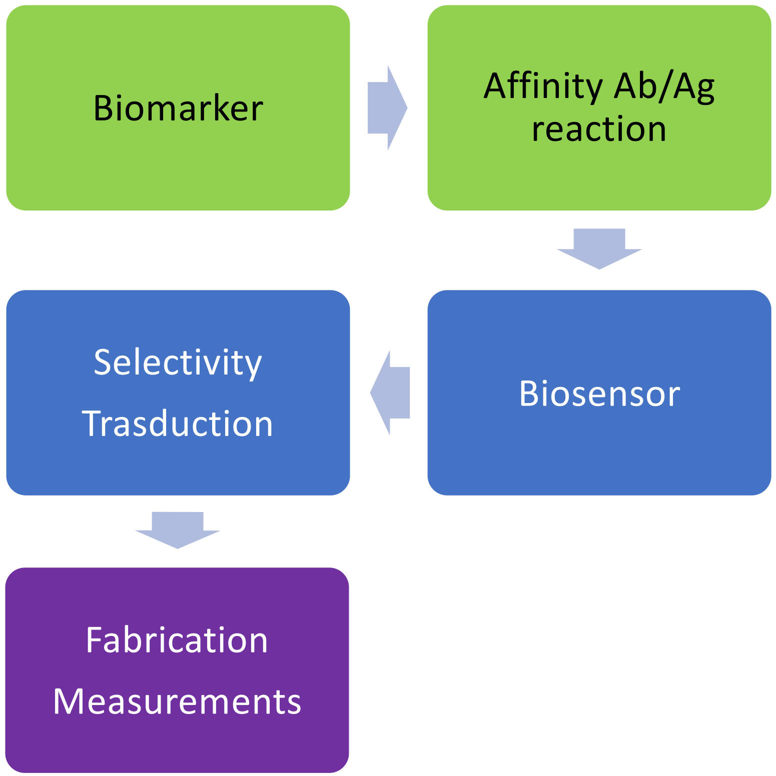
- Definition of target biomarker
- Definition of possible reactions for target detection (redox, affinity reaction, etc.)

Sensor Design

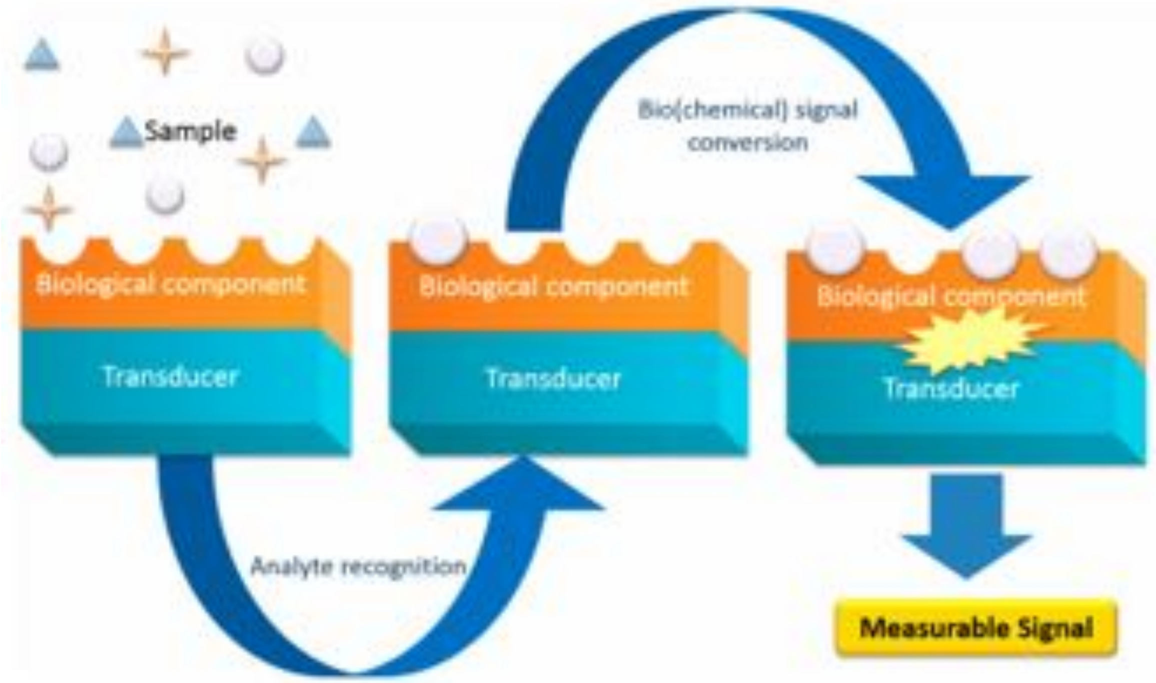
- Choose of appropriate device (EGOFET, etc.)
- Design of bio-interface (surface nanostructuration / functionalization / blocking)
- Device design

Production / integration

- Technological processing
- Basic characterizations
- Biodetection experiments

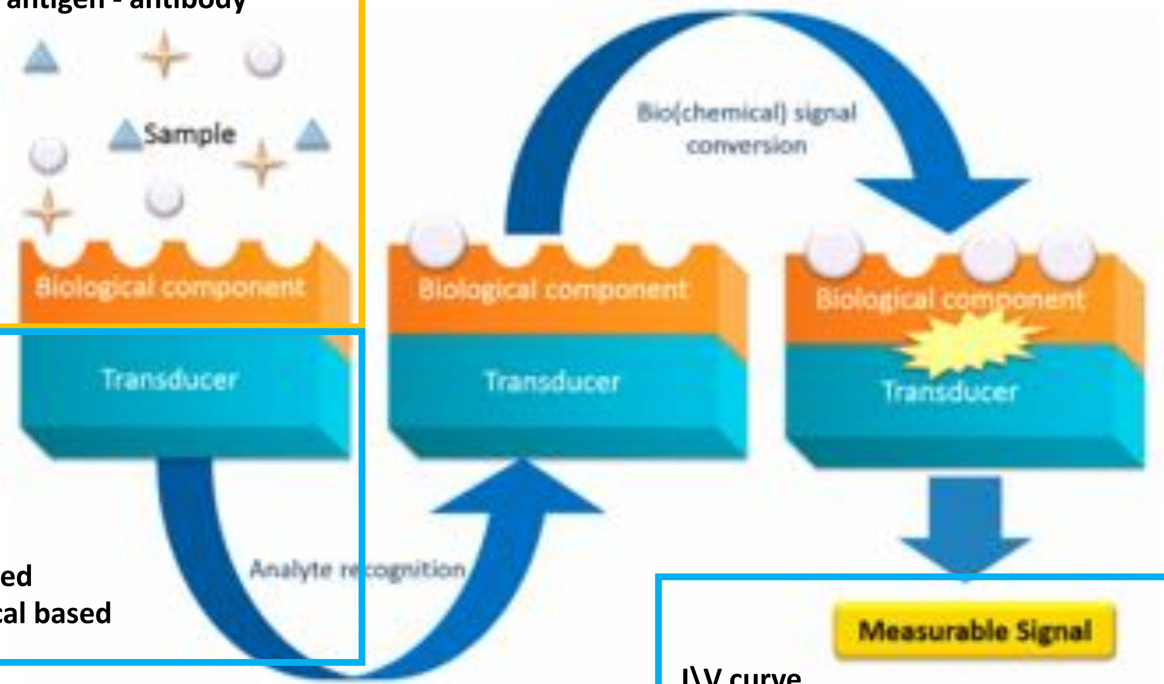


Biosensing



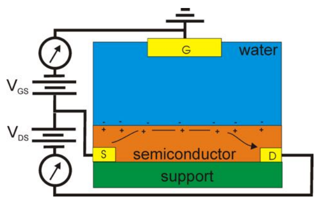
Sample
Bio(chemical) signal
conversion
Biological component
Transducer
Biological component
Transducer
Biological component
Transducer
Analyte recognition
Measurable Signal

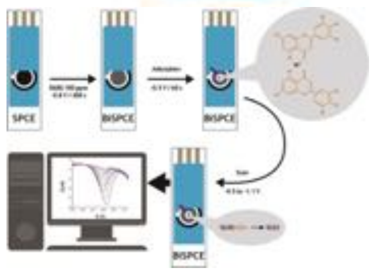
Selectivity by antigen - antibody recognition

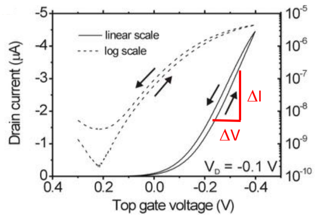


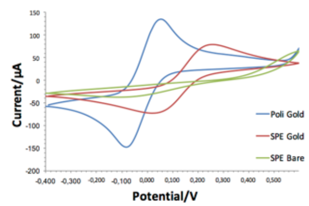
Transistor based
Electrochemical based

I\V curve
DPV - CV- EIS







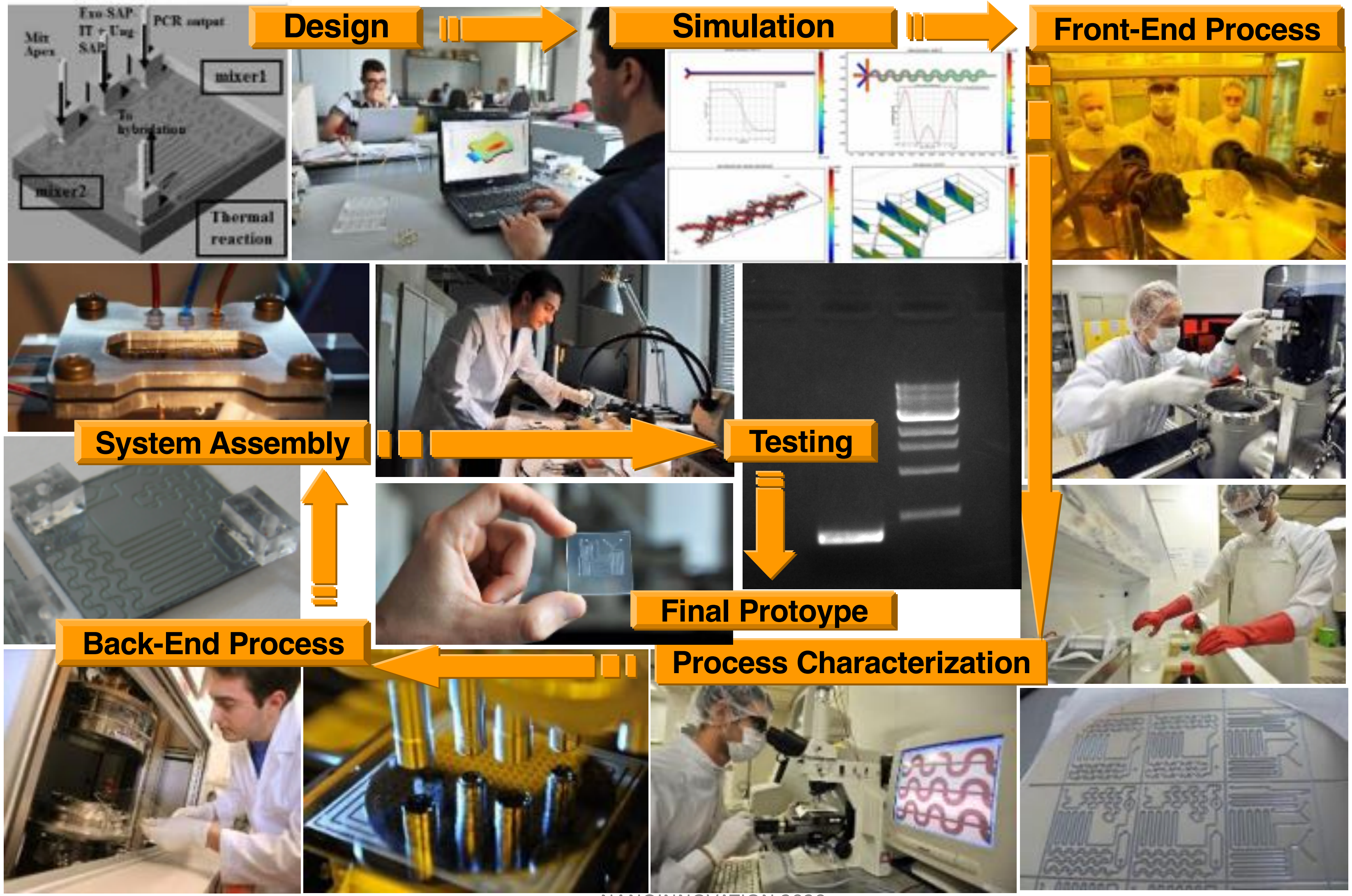


Biosensing

Devices	Technologies		Measurement Setup / Tecnhique	Target	L.O.D
	Materials	Processes			
Egofet / Gfet	Pentacene or P3HT channel Au or Pt gate	- spin-coating - photolithography -CVD -RIE - e-beam evaporator - Inkjet printing	- source meter - probe station - syringe pump	TNFα [3]	100 pM
				C-reactive protein [4]	2 pM
				interleukin-4 [14]	5 nM
				DNA and CTCs [15]	0,1pM and 100 cells/μl
Impedance-based μflow	Silicon/Polymer microfluidic device and Au electrodes		- potentiostat - EIS software - syringe pump	cell-derived micropar- ticle (MP) biomarkers [16] , [17]	10 MP/ μL
Micro Electrodes	Porous graphene + metal oxide nanostructures metal electrode + carbon/metal oxide nanostructures	-electrospinning -e-beam evaporation -electrodeposition -CVD -photolitography	- Differential pulse voltammetry (DPV) - Electrochemical Impedance Spectroscopy (EIS) - cyclic voltammetry (CV) - potentiostat	Uric acid [6]	745 nM
Macro Electrodes	Graphene/graphene oxide on GCE or SPE + metal/metal oxide nanostructures	- GO by modified Hummers method - drop casting		DNA hybridization [7]	11 fM
				glucose (enzimeless sensor) [8]	0,01 μM
				Uric acid [9]	312 nM
	Laser induced graphene + metal/metal oxide nanostructures	- Laser induced graphene on kapton - electrodeposition		glucose (enzimeless sensor) [10]	250 nM
				thrombin [11]	1 pM (in PBS) 5 pM (in serum)

DEVELOPMENT TOOLS & FACILITIES

How to develop a microsystem: combine expertise, tools and facilities



Computer aid design (CAD)

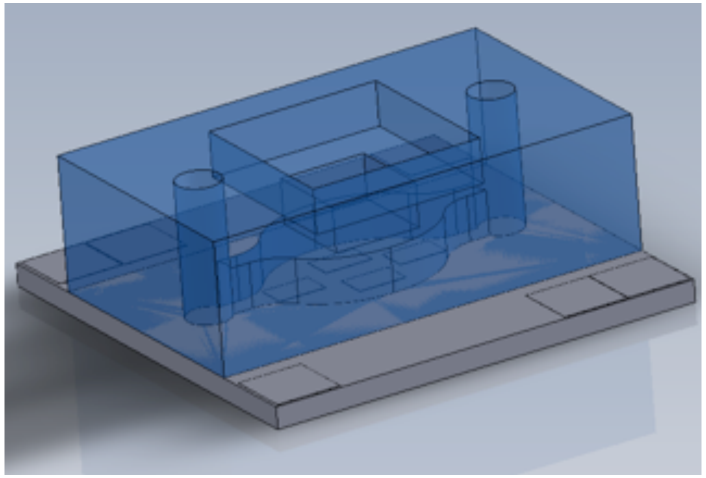
Main issues:

- vector-based graphics
- Snap functions
- multi layers design
- simple and complex geometries
- use of 2D – 3D Cartesian coordinates
- simple function to move or copy object
- Boolean operation

Rhinoceros®
NURBS modeling for Windows

QCAD.org
Open Source CAD

SolidWorks



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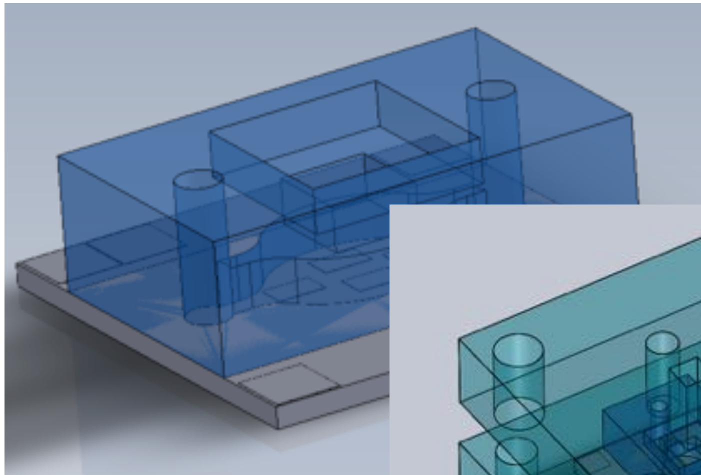
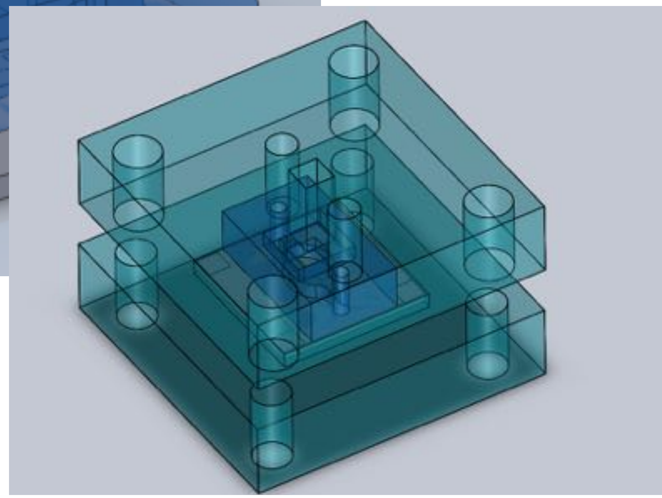
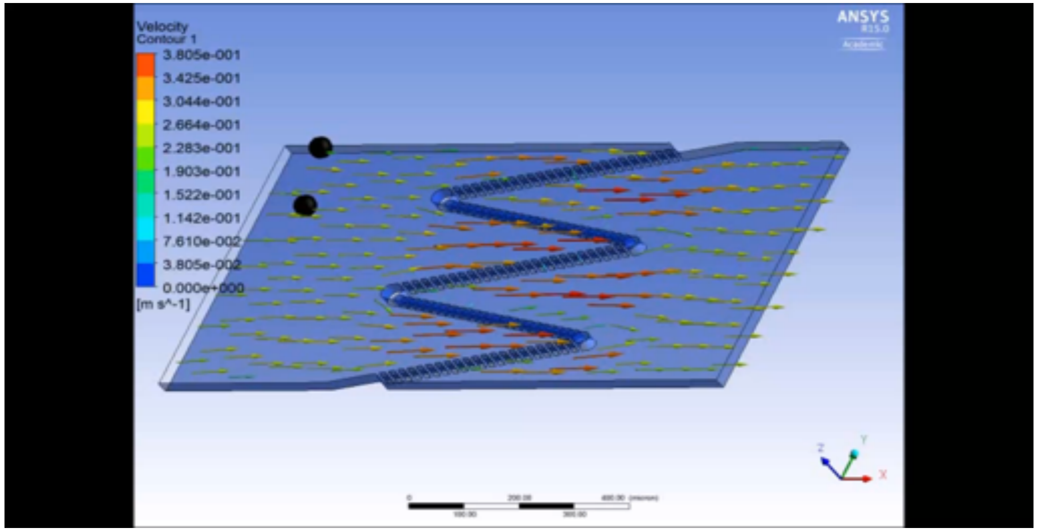




Finite elements simulation

Main issues:

- a numerical technique
- approximate solutions to boundary value problems for partial differential equations multi layers design
- Discretize and Select the Element Types (1D - 2D – 3D)
- Multiphysics features



COMSOL

Finite elements simulation

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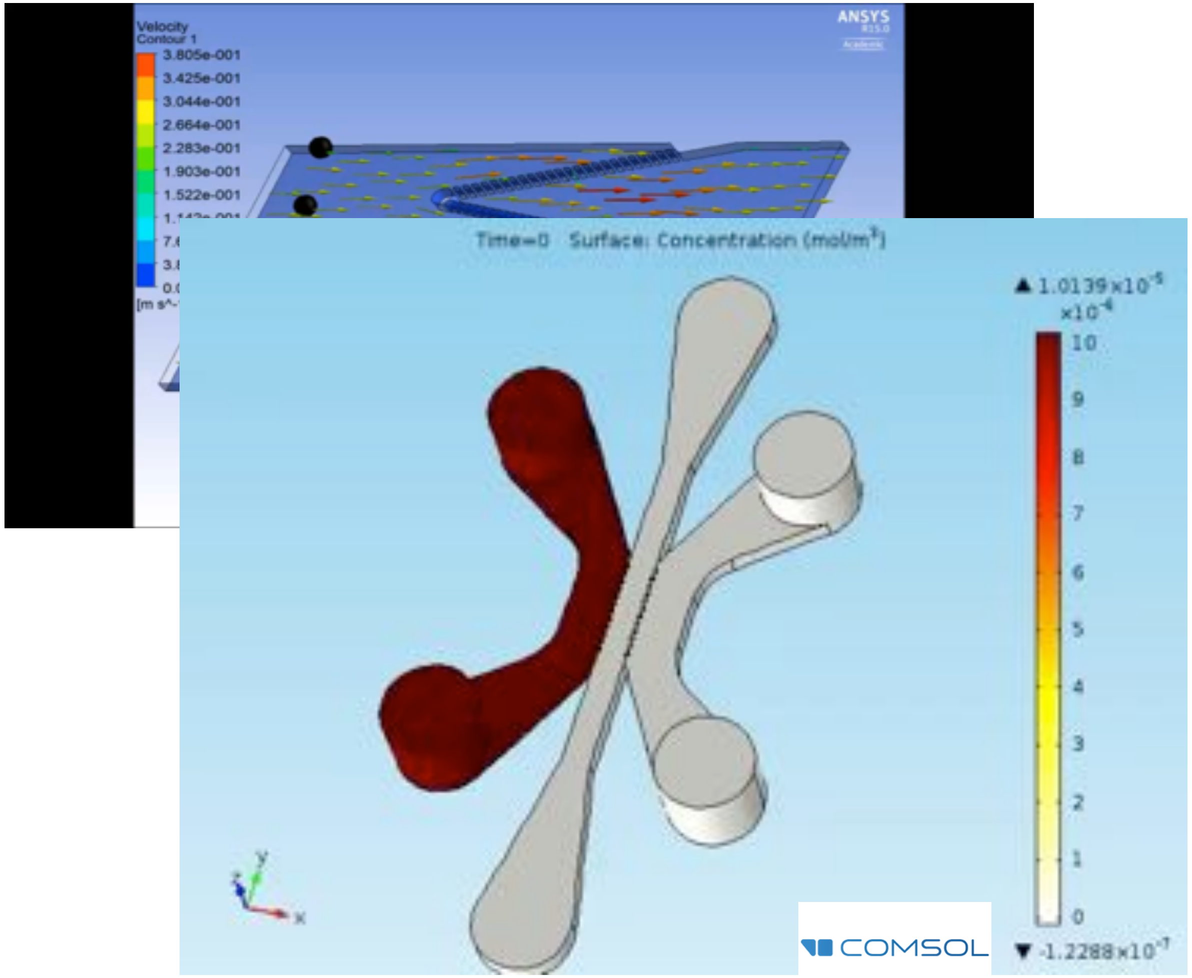
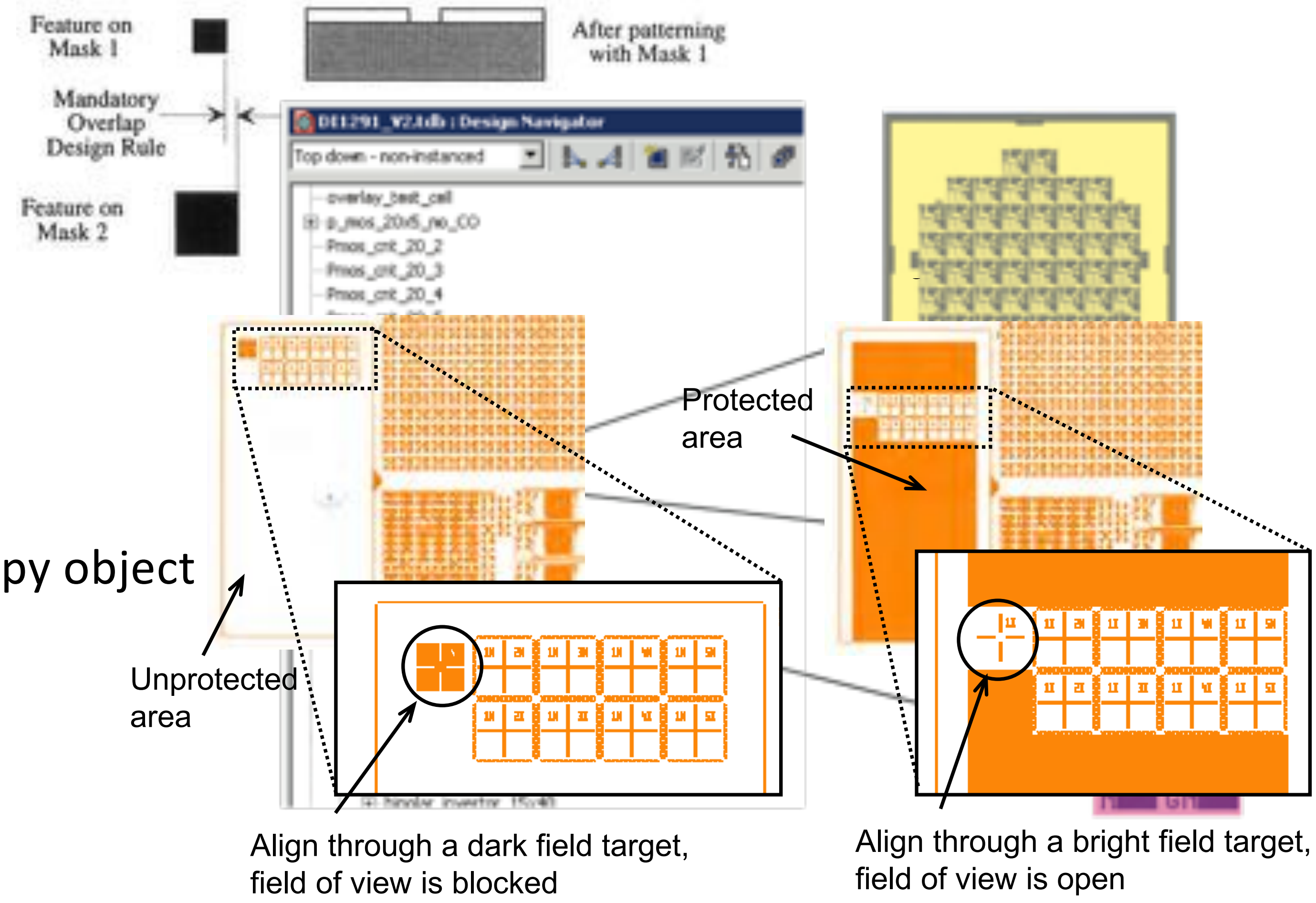


Layout editor

Main issues:

- vector-based graphics
- area design rules
- Hierarchical structures
- use of layers and arrays
- Dark / Bright field
- simple function to move or copy object
- Boolean operation



Thin Films growth technologies
- Metal evaporation systems: thermal + electron gun
- Magnetron sputtering
- Plasma Enhanced Chemical Vapor Deposition (PECVD)
- Low Pressure CVD (LPCVD)
- Silicon oxidation
- Rapid Thermal Annealing
- Electroplating
- Graphene CVD

Double-side Optical Lithography

Laser Direct Writing Lithography

Etching:
- Wet
- Dry (plasma, RIE, ICP-DRIE (Bosch® & Cryo))
- Powder Blasting

Polymer machining:
- Hot Embossing
- µ-Stereo Lithography
- µ-Injection Moulding
- Polymer 3D Ink-Jet Printing
- 3D Direct Laser Sintering
- 2 Photon Polymerization (available soon)

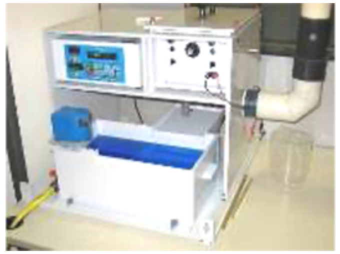
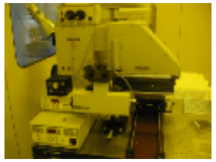
Laser Micromachining

Micro Electro-Discharge Machining

Anodic Bonding

Low and Atm. Pressure Plasma Polymerization













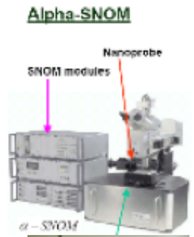




Microscopies

Optical
FE-SEM + EDX
AFM / STM
TEM/STEM
Fluorescence



Spectroscopies

UV-Vis
Micro-FTIR
Micro Raman
XRD
XPS

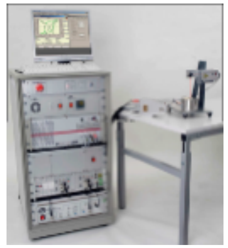



Profilometer

Contact Angle

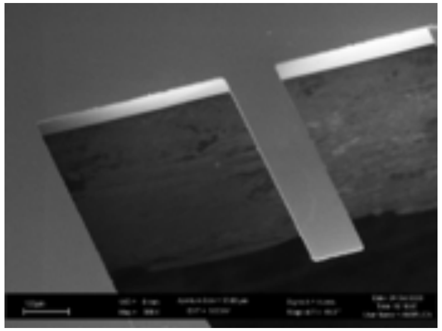
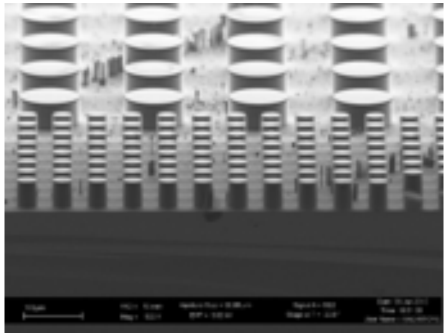
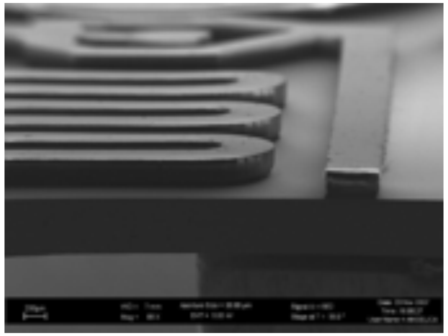
Microfluidic Characterization

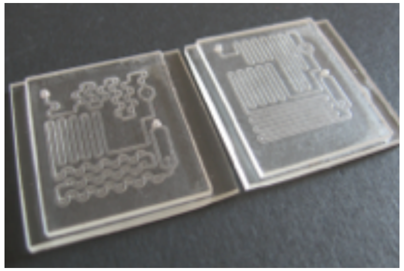
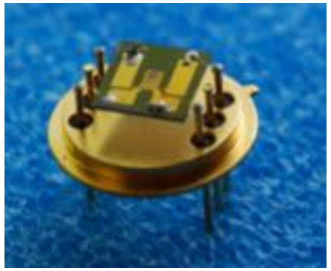
Electrical & Piezo characterizations

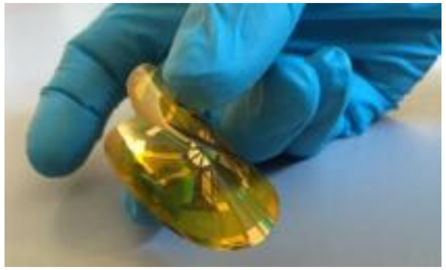
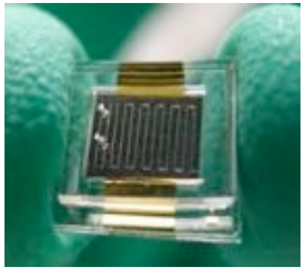



Process flow development and customized setup

- ✓ **Set-up of different technological processes and fabrication of MEMS, microfluidics and microstructures (cantilevers, microbridges, membranes, …) through Bulk Micromachining, Surface Micromachining and LIGA-like Micromachining**

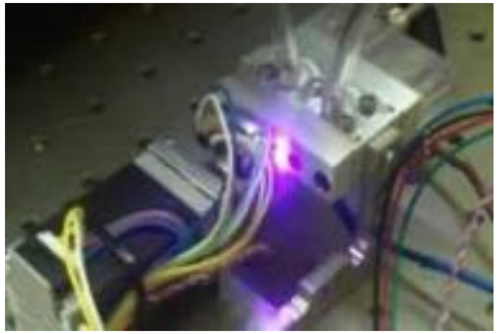





- ✓ **Engineering & fully automated customised solutions**

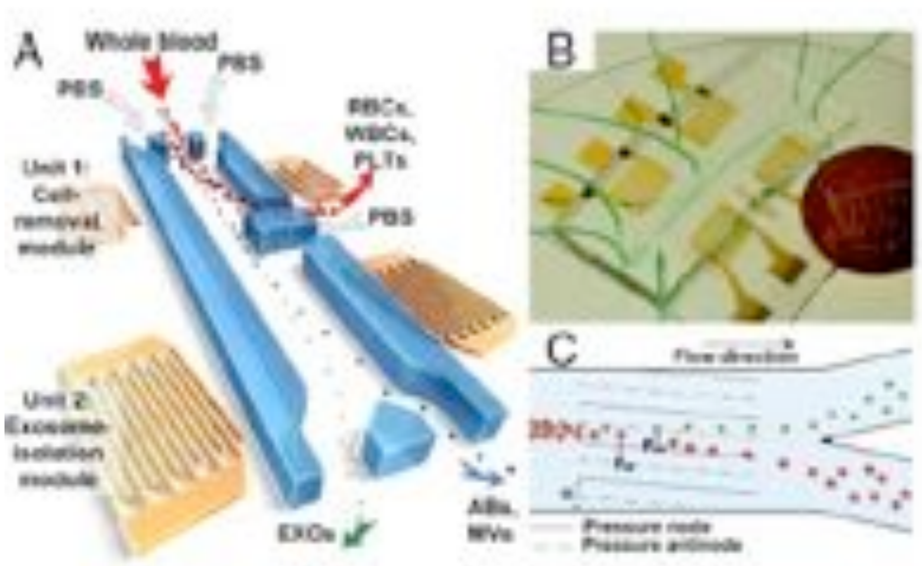


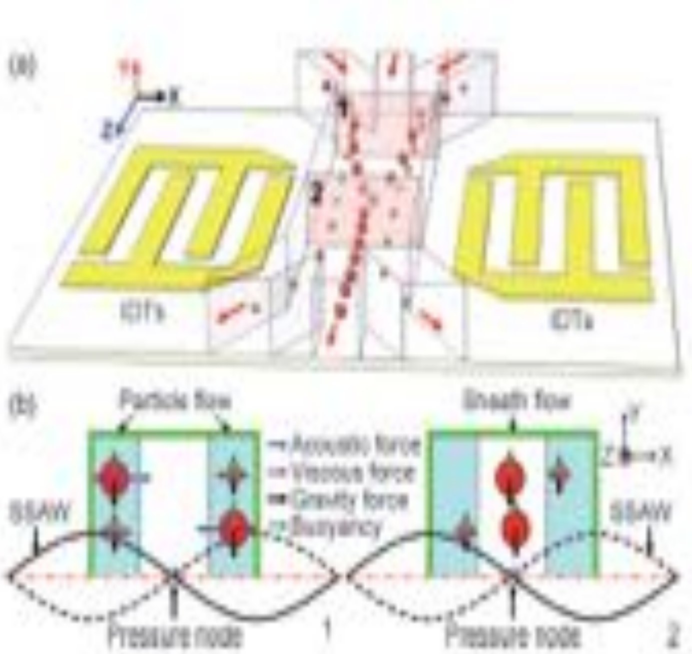


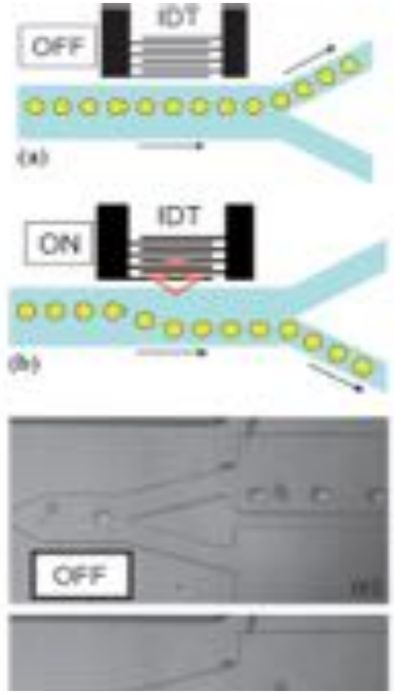



A BAW MICROFLUIDIC CHIP

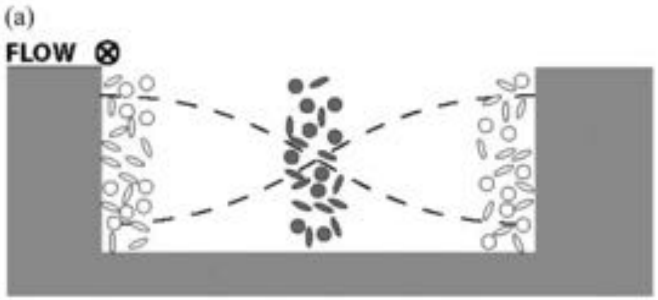
State of the art

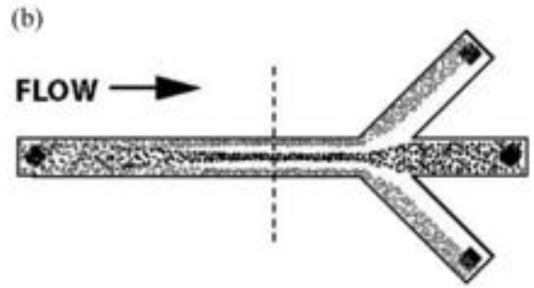






Petersson F., Nilsson A., Holm C., Jonsson H., Laurell T., *Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels.* Analyst, 2004**,** **129**, 938-943.
Shi J., Huang H., Strattin Z., Huang Y., Jun Huang T., *Continuous particle separation in a microfluidic channel via standing surface acoustic waves (SSAW).* Lab on a Chip, 2009, **9**, 3354-3359.
Franke T., Abate A. R., Weitz D. A., Wixforth A., *Surface acoustic wave (SAW) directed droplet flow in microfluidics for PDMS devices.* Lab on a Chip, 2009, **9,** 2625-2627.
Wu Mengxi, Ouyang Y., Wang Z., Zhang R., Huang P., Chen C., Li H., Li P., Quinn D., Dao M., Suresh S., Sadovsky Y., Huang T.J., *Isolation of exosomes from whole blood by integrating acoustics and microfluidics. PNAS, 2017,* ***14,*** *10584-10589.*

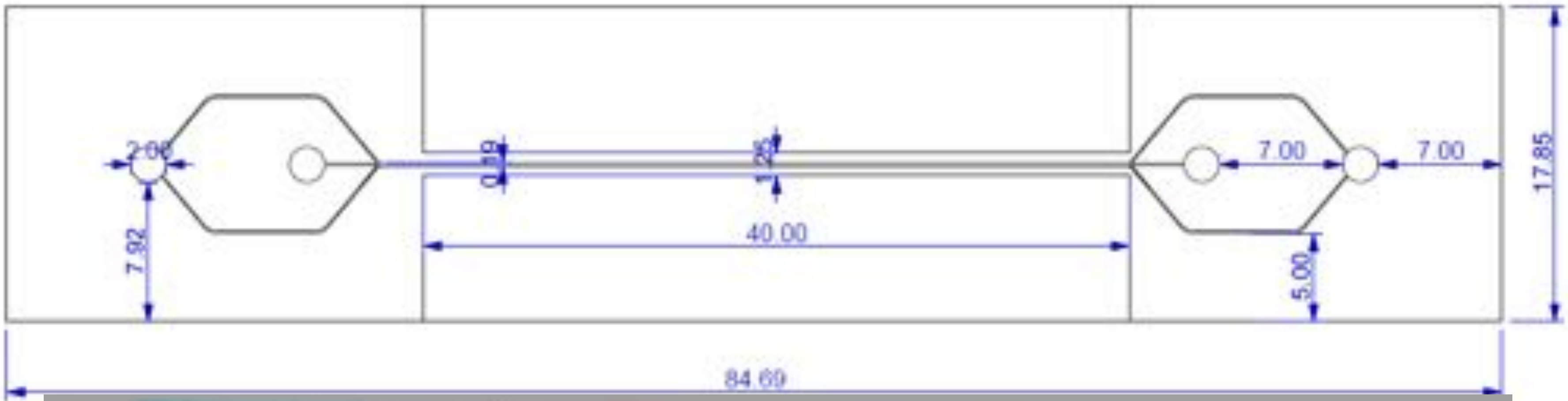




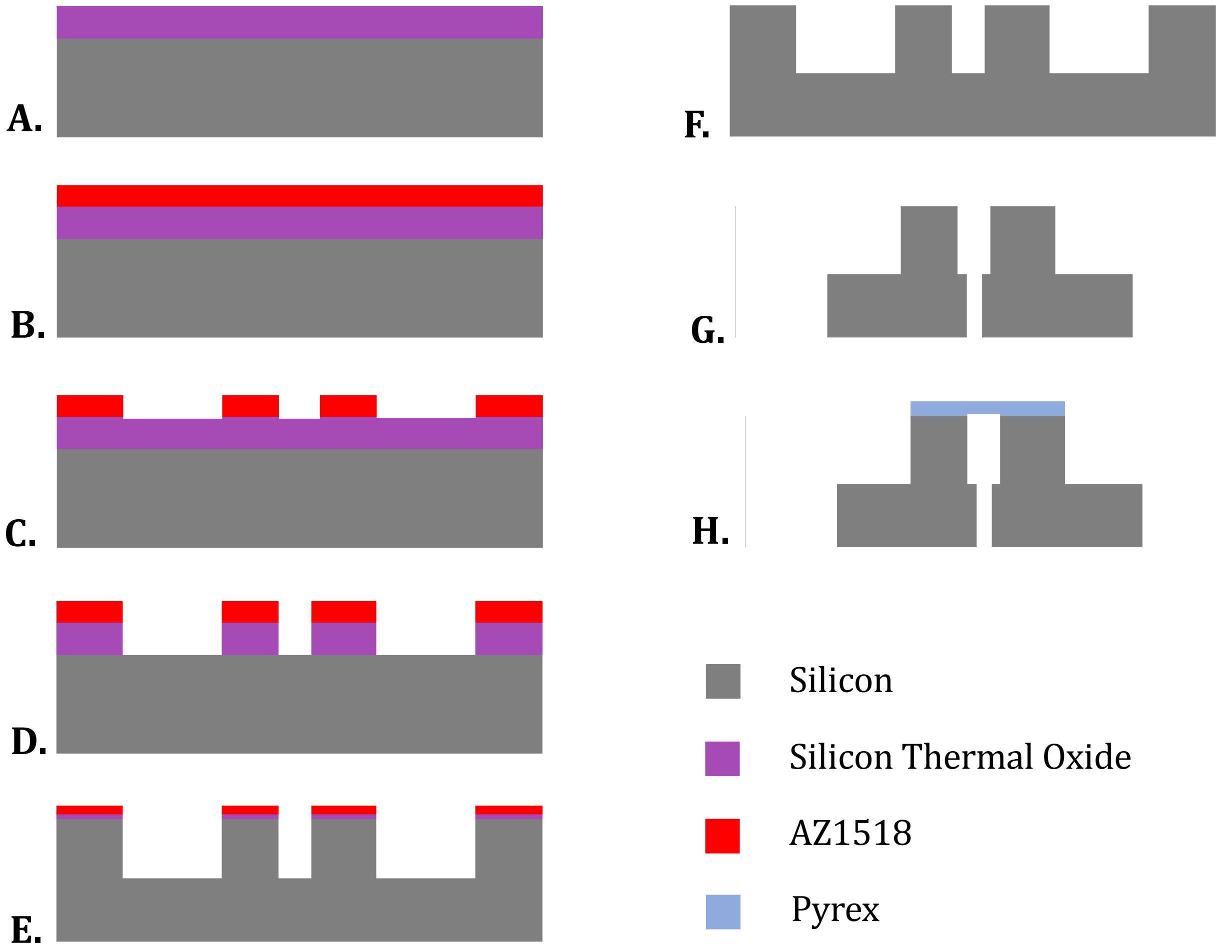
A BAW microfluidic Chip

Bulk Acoustic Wave Device design

Frequency [MHz]	W [mm]	$w_{channel}$ [µm]	h [µm] with AR = 0.5
4	1.25	190	95

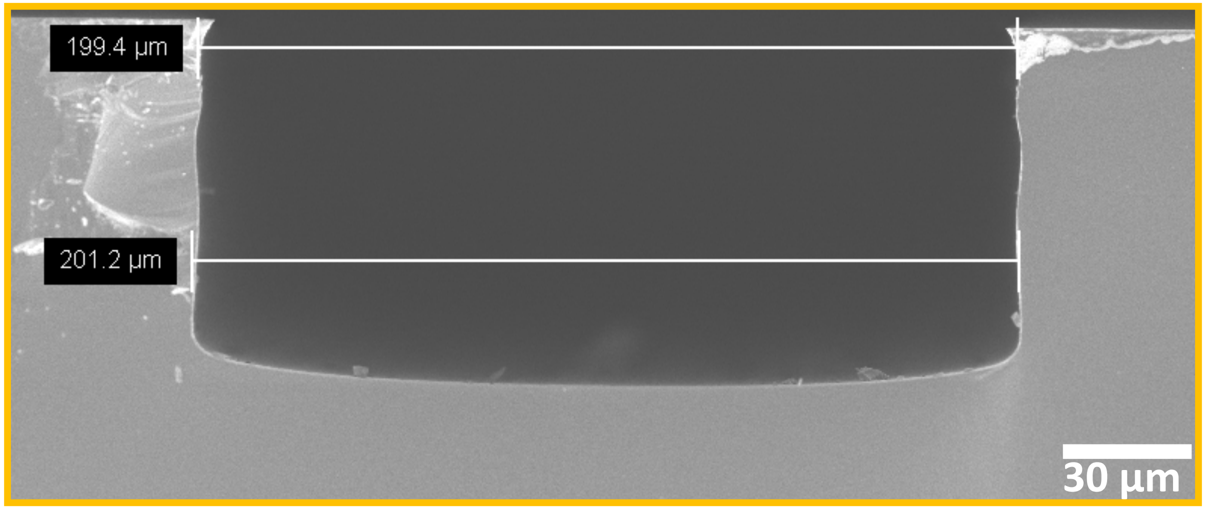


Process Flow



A. Cleaning
B. Photoresist Spin Coating
C. Photoresist Develop
D. Buffer Oxide Etching
E. Deep Reactive Ion Etching
F. Buffer Oxide Etching
G. Inlet and Outlet Laser Etching
H. Anodic Bonding

Device Characterization

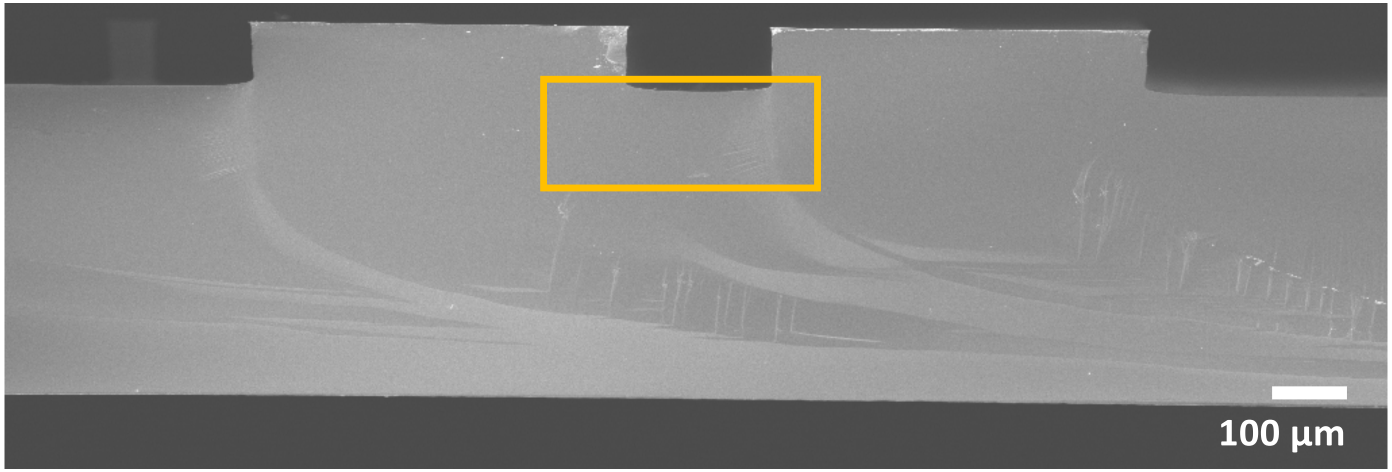


- CAD MEASURE

Channel width: 190 µm
Channel height: 95 µm
Wall width: 530 µm

- OBTAINED MEASURE

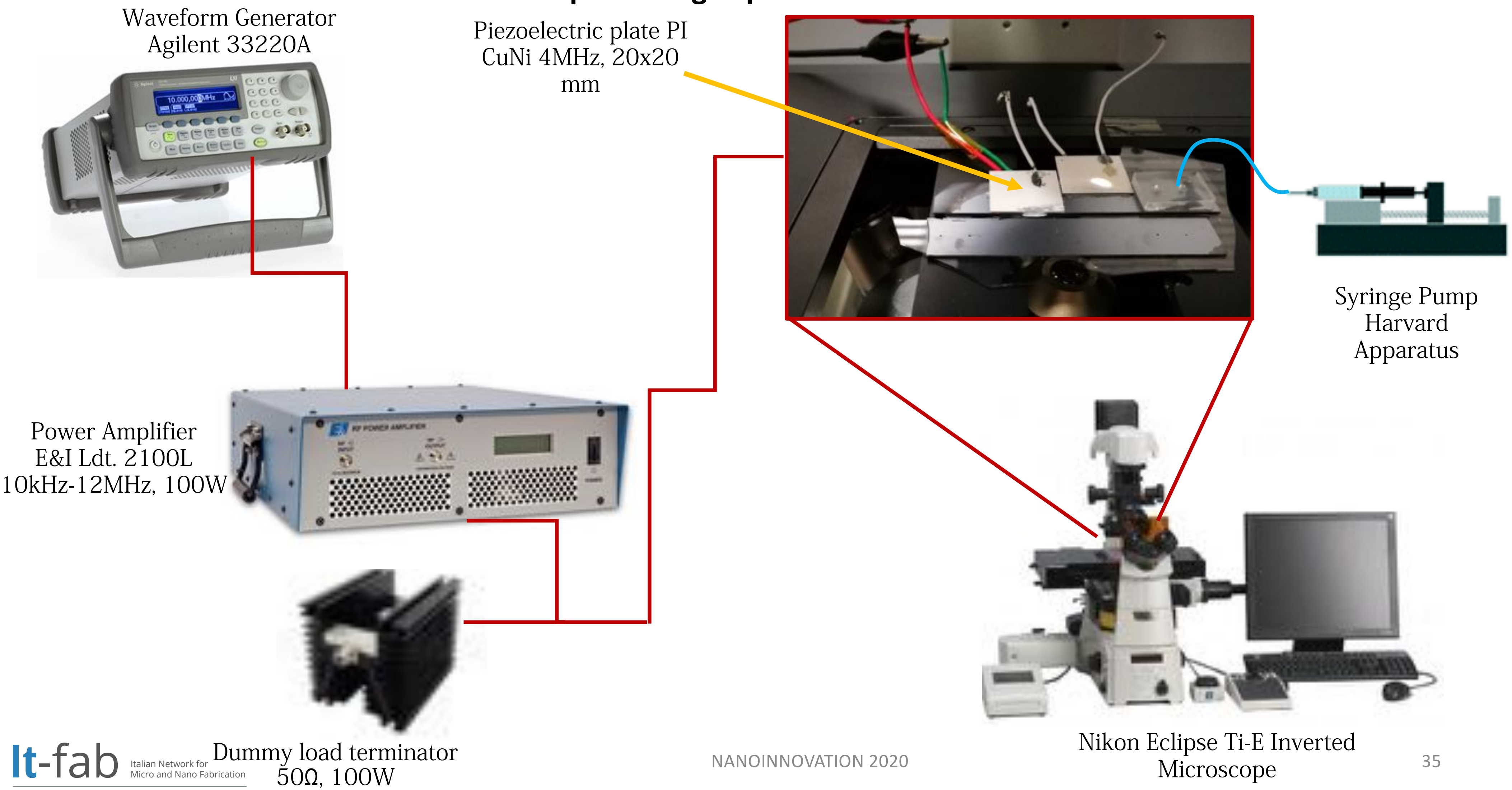
Channel width: 200 µm
Channel height: 84,5 µm
Wall width: 495 µm



Top: SEM image – zoom cross section BAW channel
Bottom: SEM image cross section BAW channel

A BAW microfluidic Chip

Set-up focusing experiment



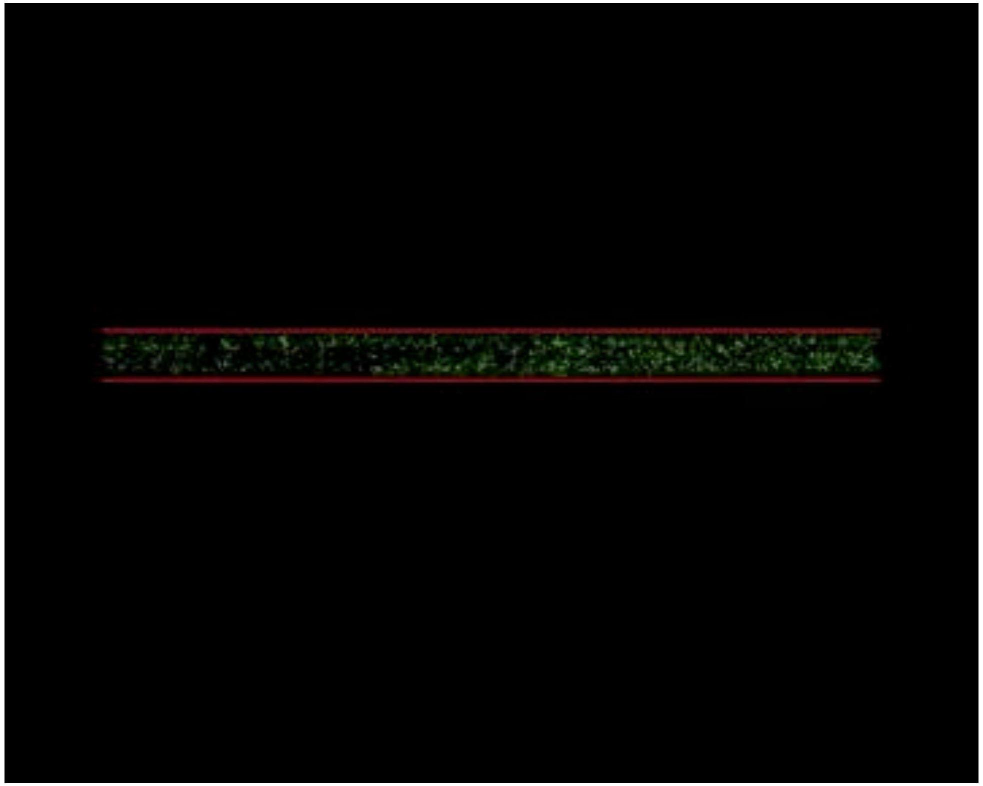
Focusing characterization Experiment 1

BAW experiment settings

- Constant values: frequency, type of signal, applied voltage, particles
- Variable values: particles’ concentration, flow rate

Costant values | **Variable values**

Frequency [MHz]	ΔV [V]	Beads' type	Concentration [particles/mL]	Flow rate [μL/min]
4.623	48	4 μm yellow-green fluorescent sulfated	5.68E+06	1
				3
				10
			1.41E+06	1
				3
				10
			5.68E+05	1
				3
				10

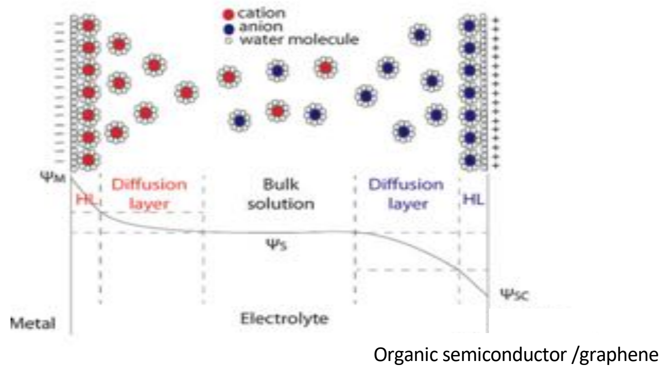


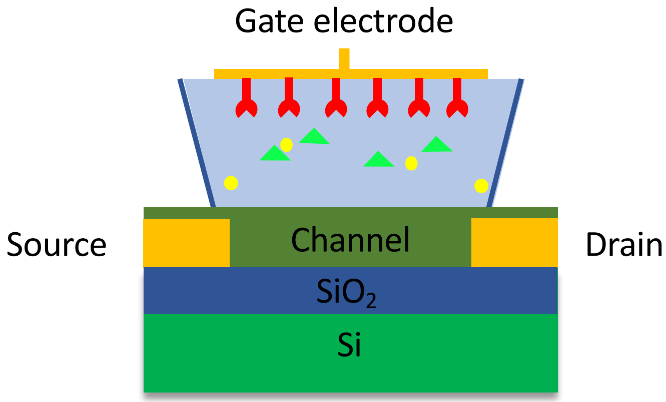
A nano- EGO-FET BIOSENSOR

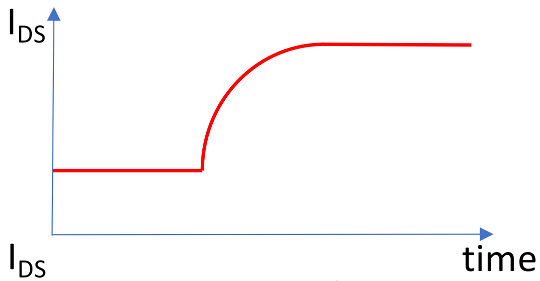
Potential variations in the electrical double layer at gate electrode/electrolyte or channel/electrolyte interfaces shift the threshold voltage of the device, thus modulating the drain current.

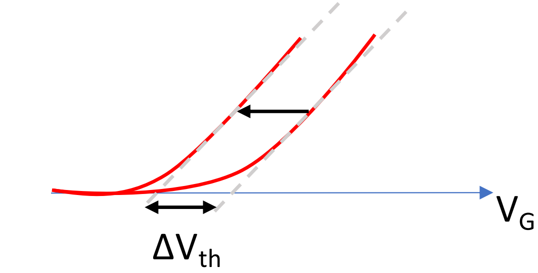
$$V_{th} \propto \psi_M, \psi_{SC}$$

$$I_{ds} = \frac{W}{L} \mu C_{DL} (V_{gs} - V_{th}) V_{ds}$$









W = channel width

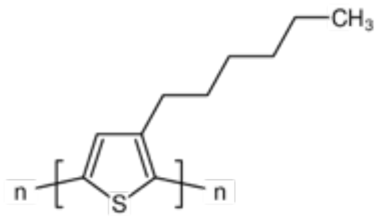
L = channel length

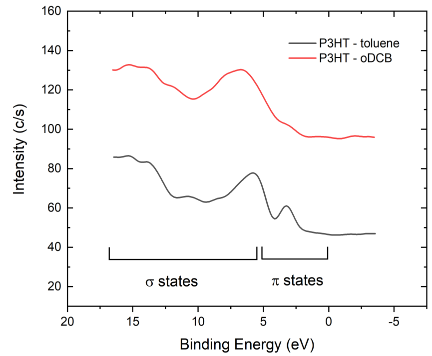
μ = charge carrier mobility

C_{DL} = electrical double layer capacitance

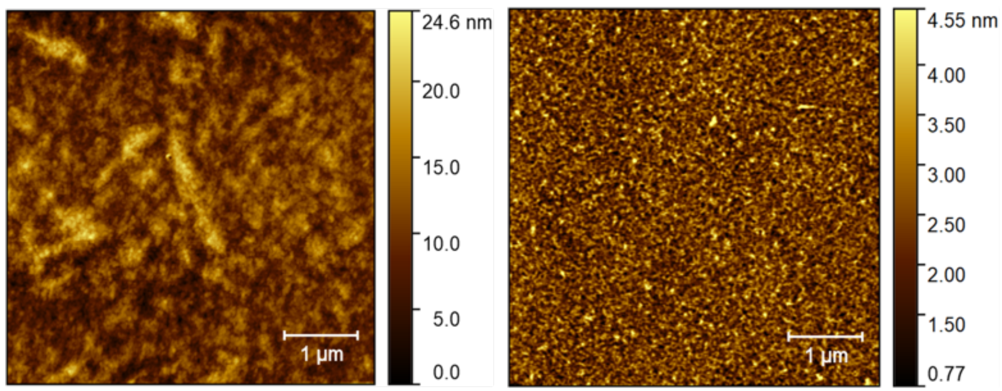
Material: Organic semiconductor

- Poly(3-hexylthiophene-2,5-diyl) (P3HT):
  - high carrier mobility ($\mu \simeq 10^{-1} \frac{cm^2}{V \cdot s}$)
  - Mw = 37kDa
  - regioregularity > 96%.





- Solvent 1,2-dichlorobenzene (oDCB)
  - Boiling point = 180 °C
  - P3HT solubility = 15 mg/ml
  - Favour $\pi - \pi^*$ stacking of polymer backbones [2]

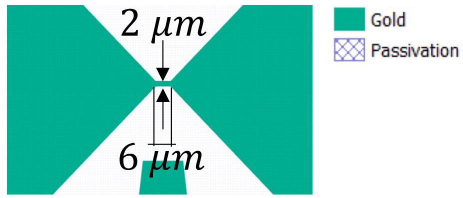


P3HT - toluene P3HT - oDCB

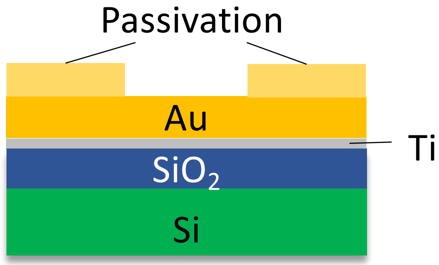
[2] M. Parmeggiani et al., "P3HT Processing Study for In-Liquid EGOFET Biosensors: Effects of the Solvent and the Surface," Sensors, vol. 19, no. 20, p. 4497, Oct. 2019.

Device fabrication

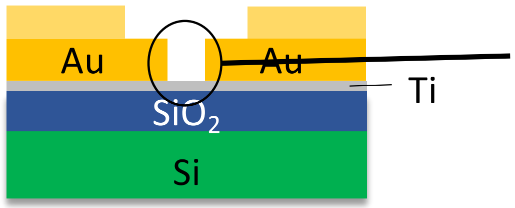
1. Au microwire fabrication
   - e-beam evaporation Ti/Au (10nm/100nm)
   - UV photolithography
   - Metal etching



2. Source/drain electrode passivation
   - Polyimide spin coating
   - UV photolithography
   - Development

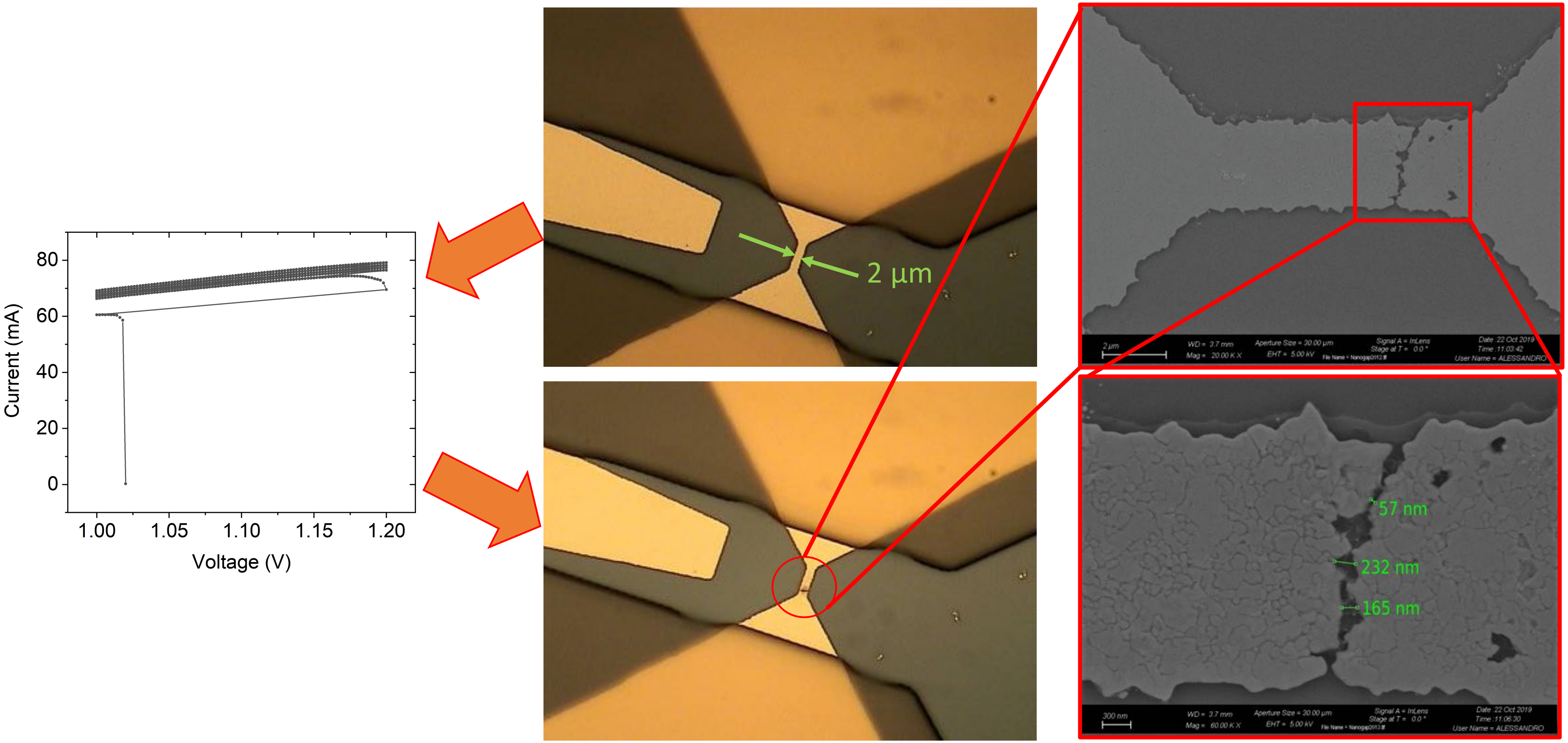


3. Electromigration by induced break junction



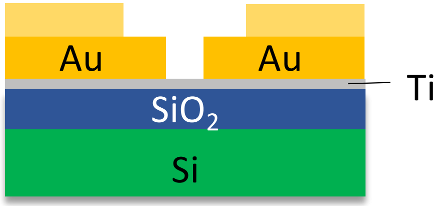
Nanogap, Ti residues may be present due to adhesion layer

Device fabrication

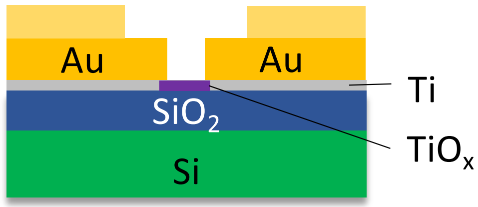


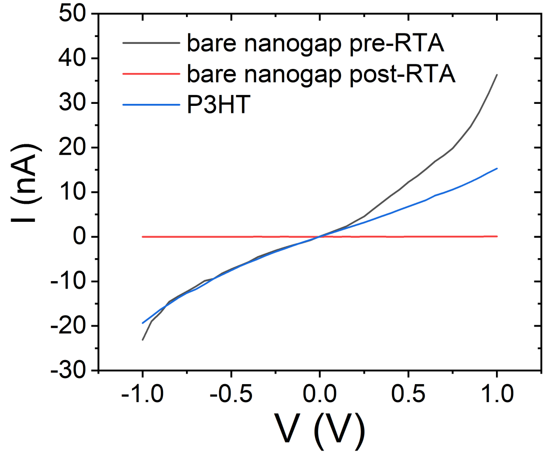
Device fabrication

3. Electromigration

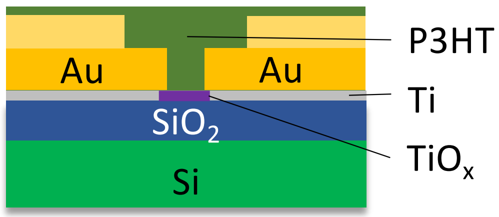


4. Titanum oxidation
   - Rapid thermal annealing in O2 at 300 °C for 20 minutes



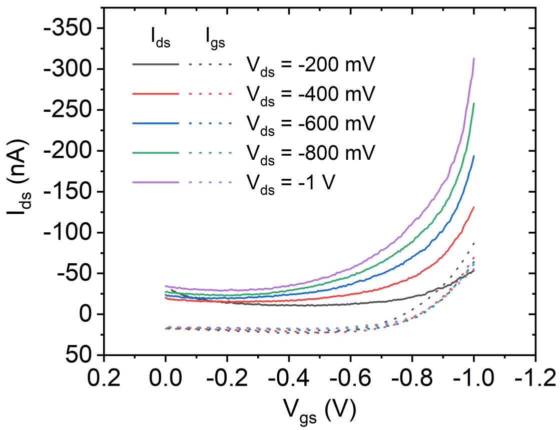


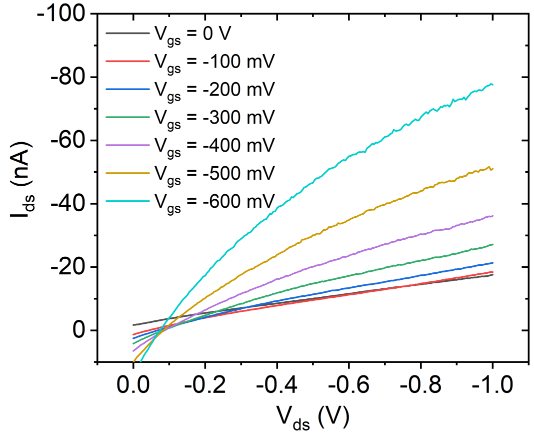
5. P3HT deposition
   - Spin coating P3HT 2.5mg/ml in oDCB, 2000 rpm for 30 s
   - Bake 1h 75 °C under vacuum

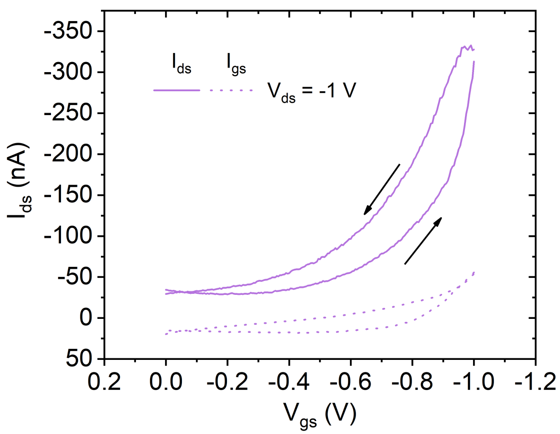


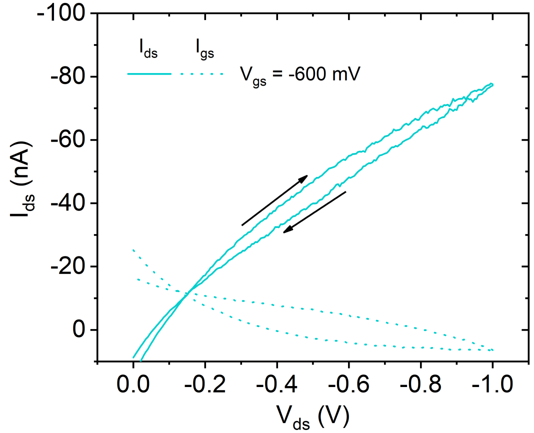
Device characterization

- Transfer and output curves measured in DI H_2O with a leak-free Ag/AgCl electrode
  - Hysteresis most probably due to trap states at P3HT/electrolyte interface
  - high leakage current may be due to small portions of Au electrodes directly exposed to water









CONCLUSION

- Biosensors and LOCs are devices high impact devices
- Micro-nano for bio mostly at research and R&D level
- Development tools & Facilities are fundamental and complex
- Expertise in different field are needed

Thank you for your attention!

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