

A fluorescence-based POCT device for immunosuppressant-drug monitoring in transplanted patients

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The name point-of-care testing (POCT) refers to the performance of biochemical, hematological, coagulation or molecular diagnostic tests **at or near the point of care**



hospital



pharmacies



primary care



home

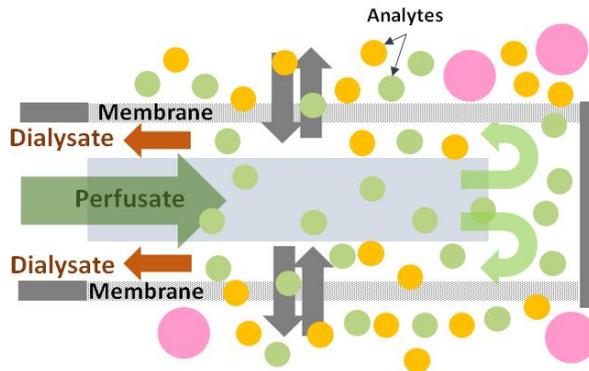
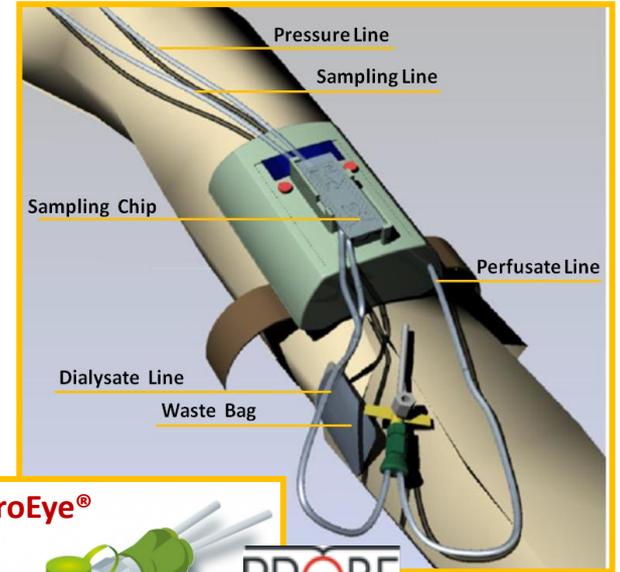
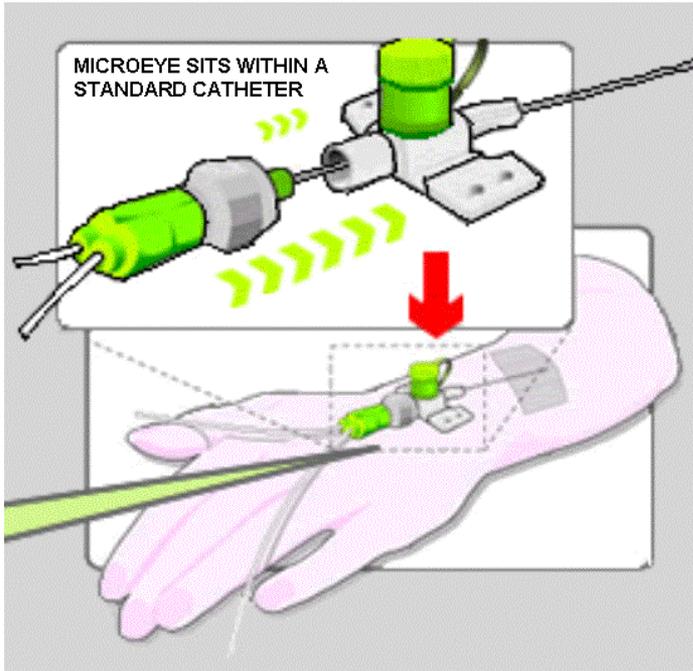


disaster area

- testing of fluidic material from the body **in immediate proximity** to the patient;
- analyses **outside a central laboratory**;
- **short turn-around-time** (TAT), meaning from sample collection to result of measurement;
- **immediate therapeutic actions**, depending on analysis results.



SAMPLING BY MICRODIALYSIS



DEFINITION OF CLINICAL REQUIREMENTS

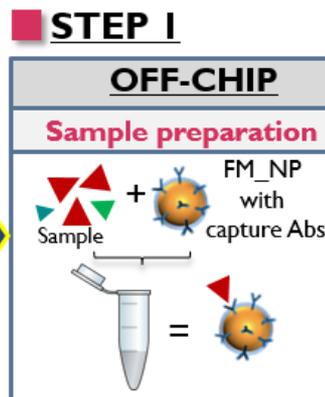
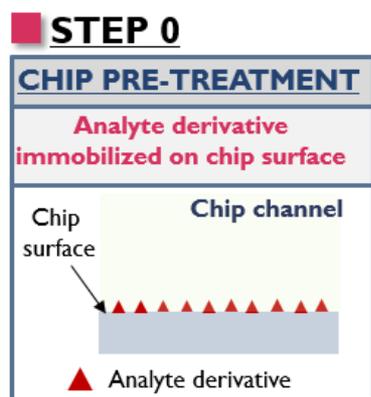
Immunosuppressant	Free fraction	LoD (ng/mL)	Relative recovery at 1 μL/min (based on in vitro tests)	LoD (ng/mL)	Dilution	LoD Nanodem (ng/mL)
Tacrolimus	1%	0.005	30%	0.0015	1:1	0.0007
Mycophenolic acid	3-17%	6-34	27.6%	1.65-9.4	1:1	0.82-4.7
Cyclosporin A	3-10%	0.6-2	6.5%	0.039-0.13	1:1	0.019-0.065

Measurement duration and frequency:

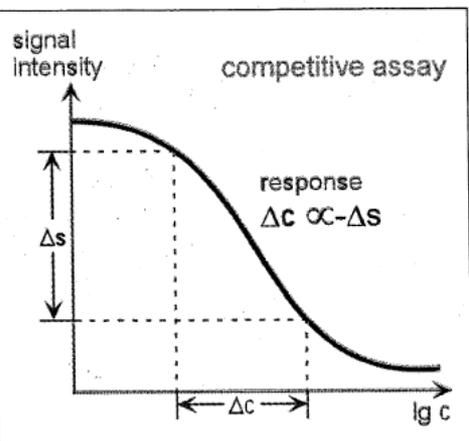
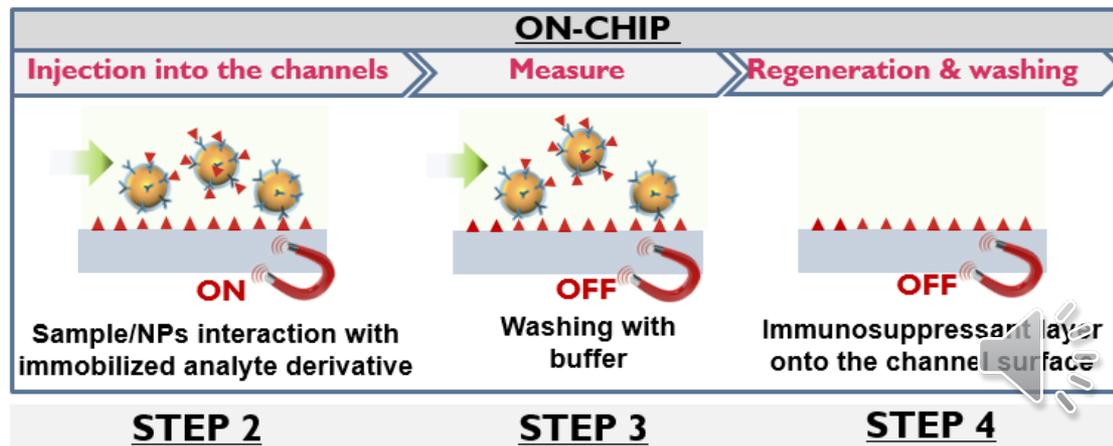
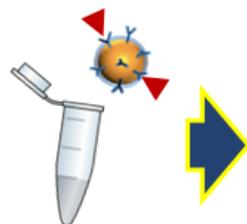
- 48 h per patient
- 1 measurement every 30 minutes

HETEROGENEOUS BINDING INHIBITION IMMUNOASSAY

THE CONCEPT

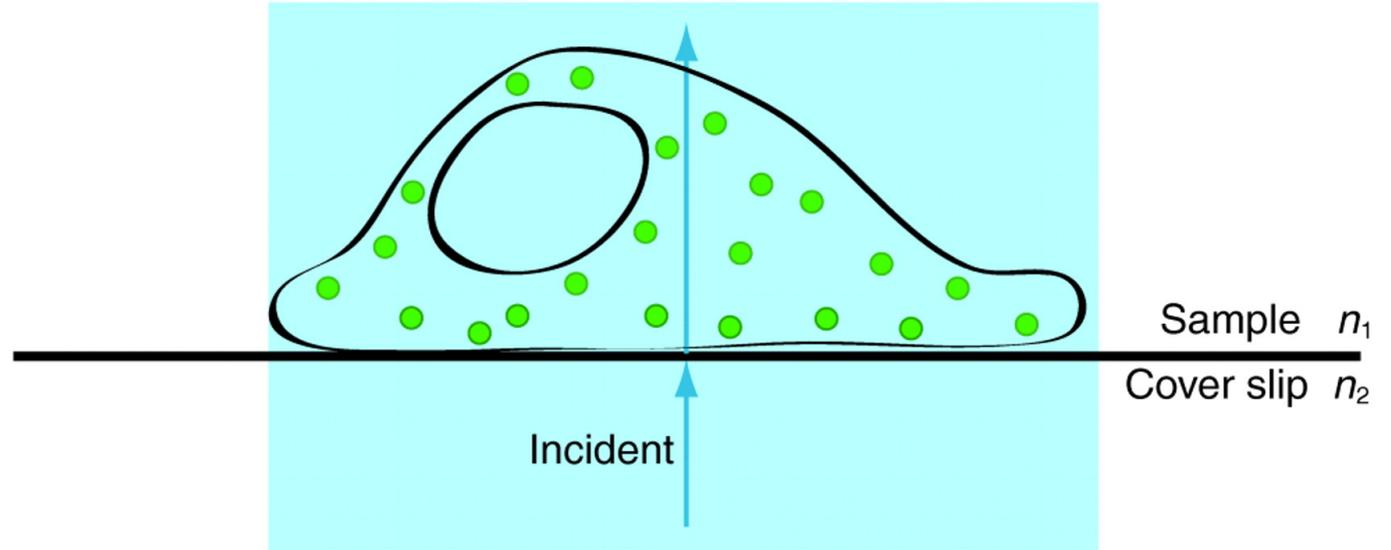


Mixed sample/NPs



THE OPTICAL CHIP

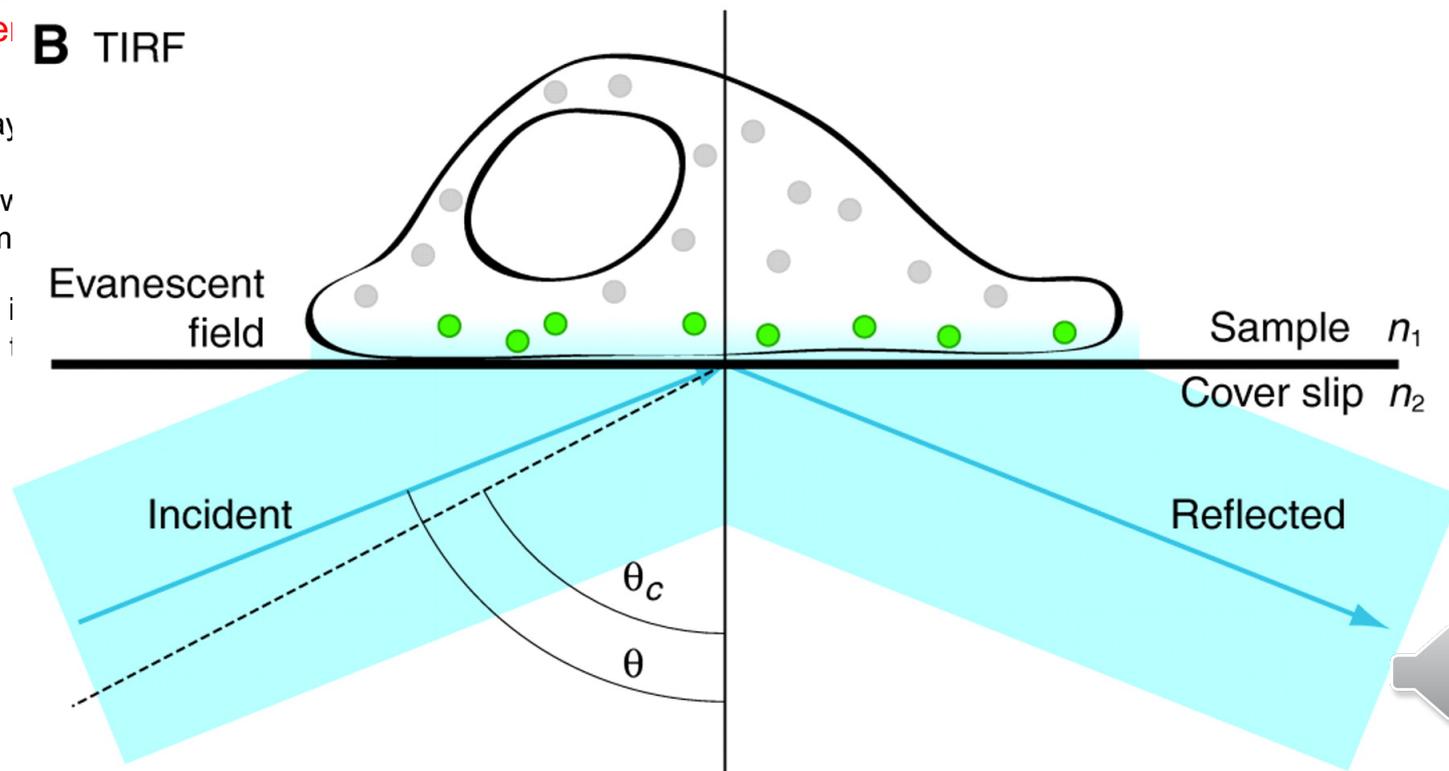
A Epifluorescence



Optical Chip
(Total Internal Reflection)

B TIRF

thin polymer layer
bonded to a low refractive index substrate
including the microscope objective
The thin film is used to excite the sample



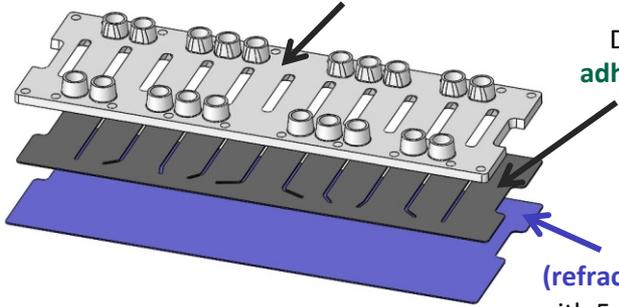
TEN - CHANNEL OPTICAL CHIP FINAL PROTOTYPE



EXPLODED VIEW

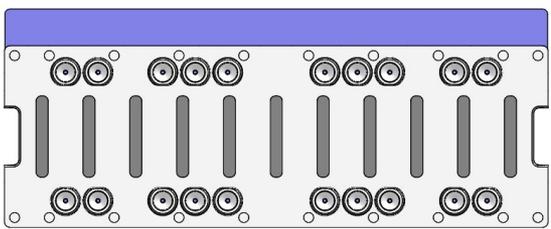
Polymer chip in Zeonex
(refractive index 1.509, thick 1.5 mm)
with 10 inlets & 10 outlets (mini Luer interfaces)

Detection channels structured in double-sided
adhesive tape (refractive index 1.49, thick 140 μm)

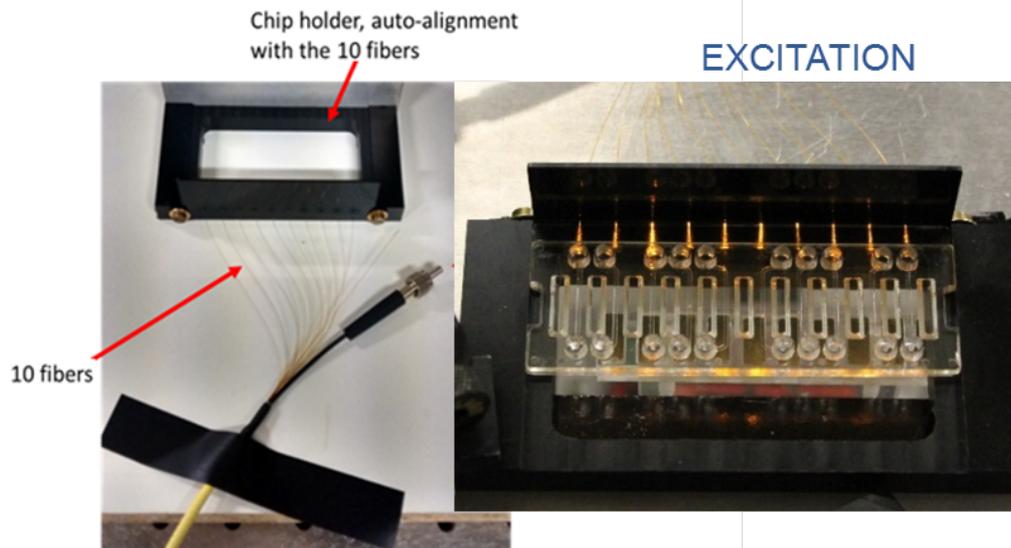
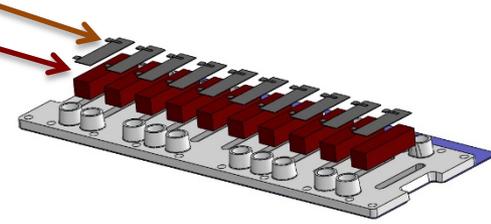
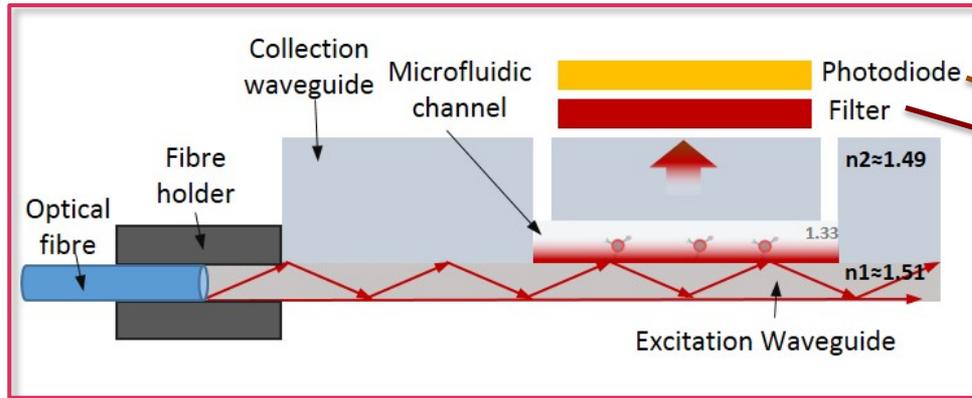


Optical foil in Zeonor
(refractive index 1.53, thick 188 μm)
with 5 mm overhang for light coupling

TOP VIEW



OPTICAL FIBRE BASED BUTT COUPLING EXCITATION SYSTEM

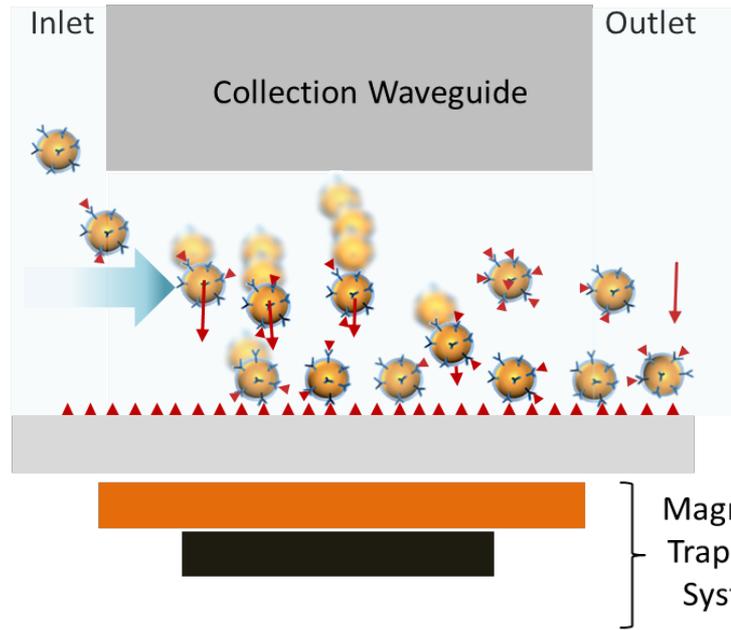


INTEGRATED MAGNETIC SYSTEM

Mixed sample / NPs



Immunosuppressant derivative



Magnetic Trapping System



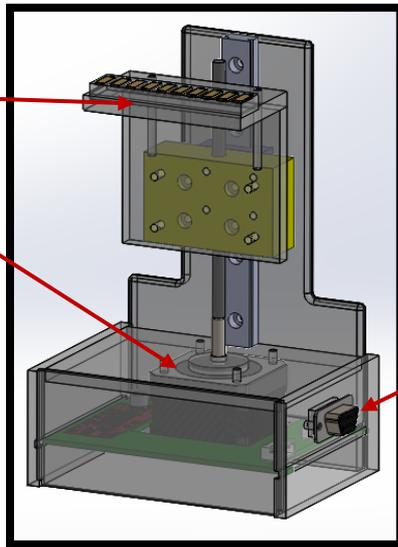
Permanent Magnet Holder

Stepper Motor

Parallel Port

INESC MN

Microsystems and Nanotechnologies



Suspension of commercial magnetic beads (Etapore®, Merck Millipore) of 300 nm diameter made of Polystyrene core, decorated with ferrite nanograins (ca. 8-10 nm size).

The magnetic beads are functionalized with carboxylic groups (-COOH) on the surface for the immobilization of antibodies.

The core of the magnetic beads is doped with the hydrophobic boron-diyromethene BODIPY-641 dye, (UCM).

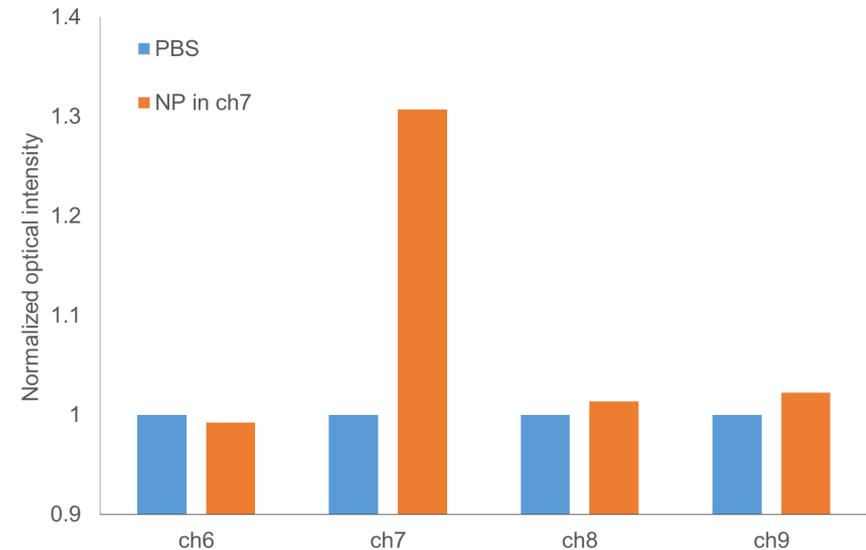


Reproducibility of the acquisition and detection of the signal as a function of the microfluidic chip insertion and positioning

Stability Test

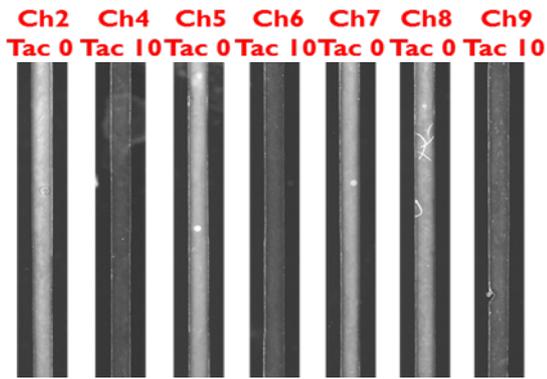
	Meas. 1 (% full scale)	Meas. 2 (% full scale)	Meas. 3 (% full scale)	Meas. 4 (% full scale)	Meas. 5 (% full scale)	Average	St. Dev.
Ch 3	4.42	4.60	4.41	4.41	4.39	4.44	0.086
Ch 4	5.87	5.80	5.86	6.13	6.08	5.95	0.145
Ch 5	6.47	6.32	6.30	6.77	6.67	6.50	0.209
Ch 6	4.92	4.81	4.86	5.11	5.06	4.95	0.130

Cross-talk evaluation between microfluidic/optical detection channels



Nanodem 10 channels chip was modified with MPA via carbodiimide crosslink method.

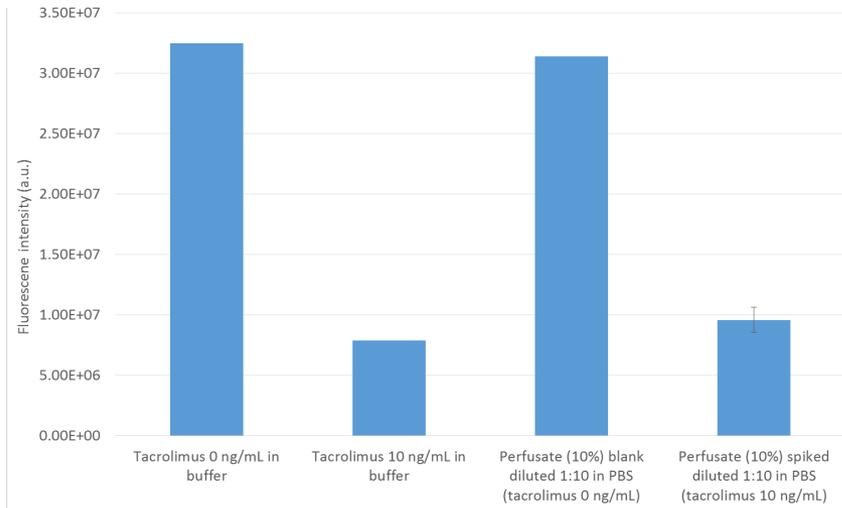




- **Regeneration efficiency** (guanidinium hydrochloride 6 M pH 1.5): **97%**
- Fluorescence intensity **reproducible** among the different assay cycles with a CV% $\leq 10\%$ on all the channels.

Adapted to perfusate:

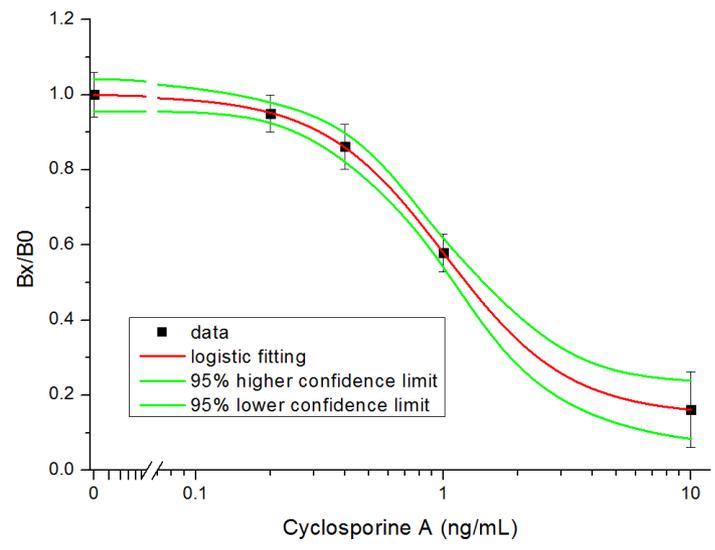
10% perfusate Lipofundin® diluted 10 times in buffer, alone or spiked with tacrolimus (10 ng/mL)



Mycophenolic acid assay	Assay time	LOD (ng/mL)	CV%
Assay with anti-IgG*	1 h	0.72	10%
Assay with magnetic particles, no magnet	1 h	1.4	13%
Assay with magnetic particles, magnets array	20 min	1.0	7%



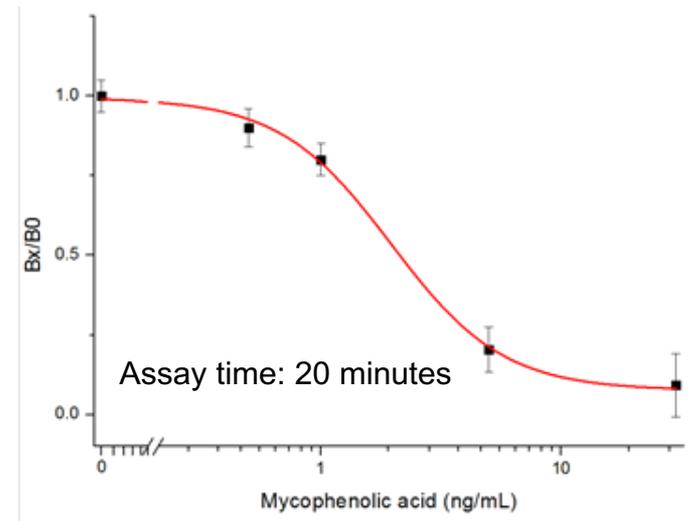
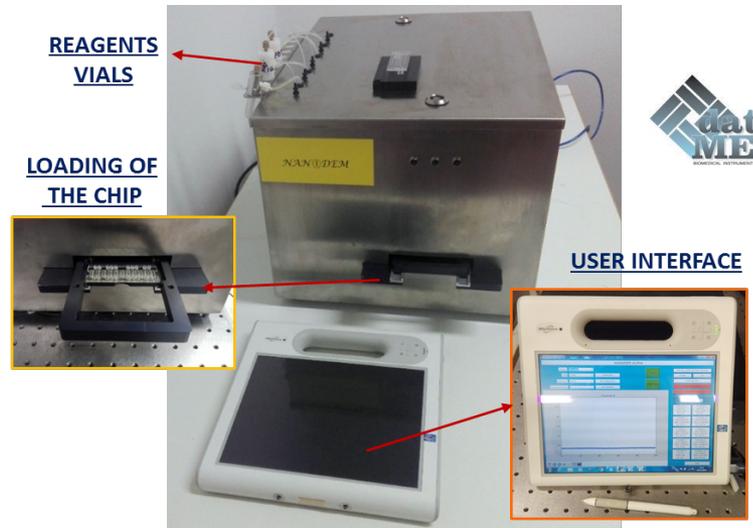
Cyclosporine A and Mycophenolic acid assay in the final prototype



Cyclosporine A in 20% Lipofundin

Logistic fitting curve ($A1=0.999$, $A2=0.146$, $x0=1.02$, $p=1.75$)
 LOD 0.48 ng/mL
 Minimum detectable concentration (MDC): 0.2 ng/mL
 Reliable detection limit (RDL): 0.21 ng/mL
 CV% on the blank: 6%
 Average CV%: 6.5%

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 Institut für Klinische Chemie und Pathobiochemie



Mycophenolic acid in 20% Lipofundin

LOD: 0.79 ng/mL
 CV% on the blank: 5%
 Average CV%: 7%

Assay time: 20 minutes



