“Short-Term Mobility” 2009

Scientific report on the research activity developed at the Institut für Ökologische Chemie, Helmholtz Zentrum München, Germany during the period 15 June – 8 July 2009

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TITLE:
Optical platform for the simultaneous detection of inflammation markers

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Summary

1. Introduction

Optical biosensors find wide applications in the development of a point of care testing (POCT). In this kind of applications, differently from genomics and proteomics where thousands of sensing spots are monitored simultaneously, it is often necessary to measure a limited number of parameters in order to diagnose a pathology or to follow the efficacy of a therapy. The basis of POCT devices is the need of physicians to have a fast and reliable response to formulate the right diagnosis or to decide the correct therapy, avoiding to deliver the samples to the central laboratories and to wait for a period of time, generally several hours long, to achieve the results of the analysis. The timeliness in a correct diagnosis can be also essential in many cases, such as the discrimination of viral and bacterial sepsis in intensive care patients or the fast identification of the origin of infections. In this case, the simultaneous measurements of analytes such as C reactive protein (CRP), procalcitonin (PCT), tumour necrosis factor α, myeloperoxidase, interleukine-6, interleukine-8, interleukine-10 and neopterin can be extremely important for physicians.

The five-year integrated project "health CARE by biosensor Measurements And Networking" (CARE-MAN) has the aim to develop an intelligent and fully automated optical diagnostic device on a modular technological system. It will combine successful transduction principles, biochemical recognition methods and communication capabilities to allow a multi-parameter measurement characterizing diseases defined by doctors and needs in hospitals. In particular, the realisation of POCT device is the final goal of the project.

Within this project a multichannel array for chemical and biochemical parameters is under development and in particular the geometry and optical properties of the PMMA chip were studied and taken into account for the chip realisation. Then, also studies on the chemical protocols in order to improve the yield of the immunoassays were performed. In particular, during this "Short-Term Mobility" experience, tests on two main markers for sepsis (CRP and PCT) were performed. Moreover, the first-prototype instrument performances were tested and the chemical protocols for the immunoassay procedures were defined thanks to the exchange with the host Dr. Petra Krämer.

2. The optical device

The Capillary Array Interrogation (CAI) instrument (Fig. 1) was developed during the evolution of the 5-year integrated-project CAREMAN, with the collaboration between IFAC-CNR (Firenze) and the company Datamed (Milano) and the laboratory characterisation on the first automated prototype was the aim of this "Short-Term Mobility" experience, exploiting the experience of the host, partner in the CARE-MAN project, in the area of immunoassay.

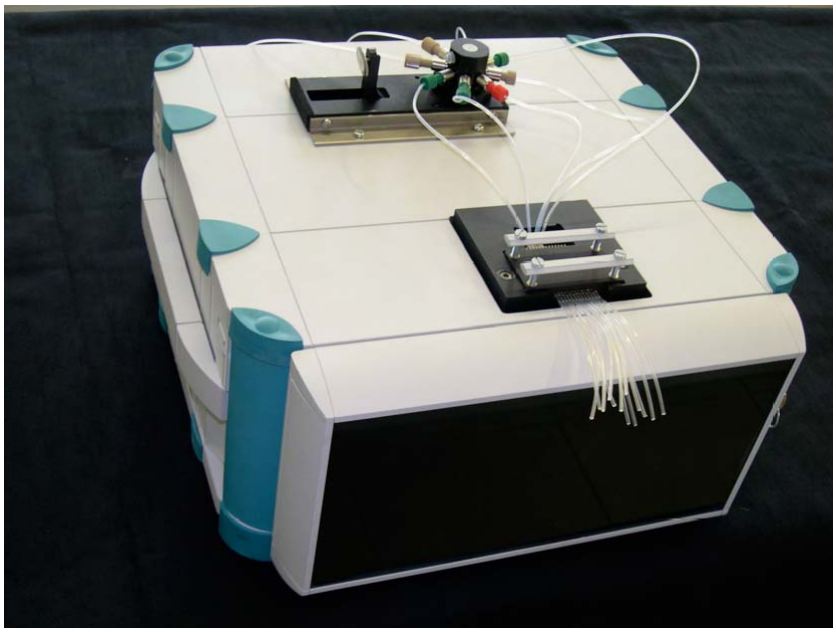

Fig. 1. The Capillary Array Interrogation (CAI) instrument

The heart of the platform is an optical biochip constituted by a two-piece polymethylmetacrylate (PMMA) chip, with 13 microchannels through which the analysed sample flows (Fig. 2). It is constituted by a transparent cover that allows the light excitation and the fluorescence collection and by a black bottom that strongly reduces the background due the scattered light, coming directly from the optical source. The cross section of each microchannel is a rectangle 600 μm wide and 100 μm high. The chip was produced by injection moulding by the German company MicroFluidic ChipShop GmbH, on the basis of the IFAC and Datamed design.

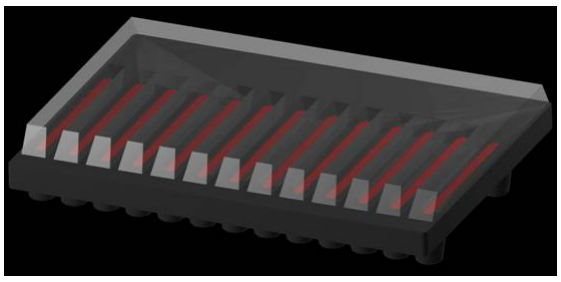

Fig. 2. 13-microchannel chip in PMMA

The sensing layer, where the immunochemical reaction takes place, is located on the bottom side of the upper piece of the PMMA chip. The exciting radiation, perpendicular to the sensing layer, comes from a laser diode emitting at 635 nm, properly filtered with a band pass filter at 635 nm and focused by means of a cylindrical lens. The emitted fluorescence, which comes from the sensing layer where the specific biologic interaction takes place, is mainly coupled to the PMMA cover of the chip and the working principle of the optical interrogation unit is schematically represented in Figure 3.

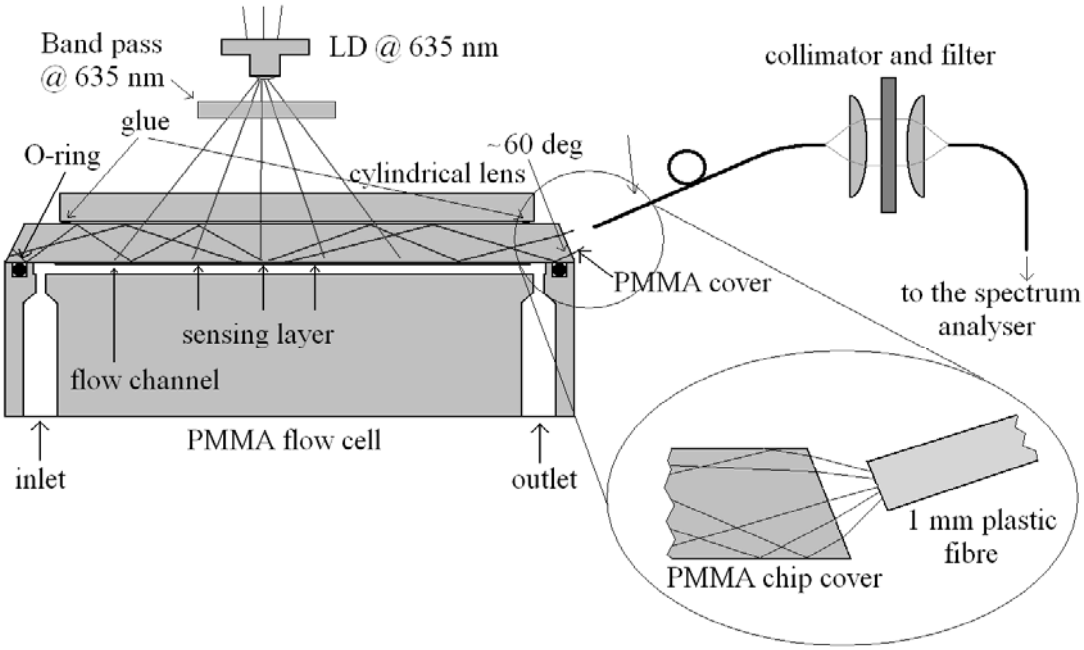

Fig. 3. Working principle of the optical interrogation unit

This occurs thanks to the fluorescence anisotropy, which is exhibited whenever the fluorophore is located at a distance of a medium interface of the order of the wavelength. The fluorescent signal is laterally collected by a single plastic optical fibre and coupled to a cooled HAMAMATSU photodiode. A high-pass filter, cut on at 650 nm, takes care to cut the light coming from the source and scattered by the PMMA chip (Fig. 4).

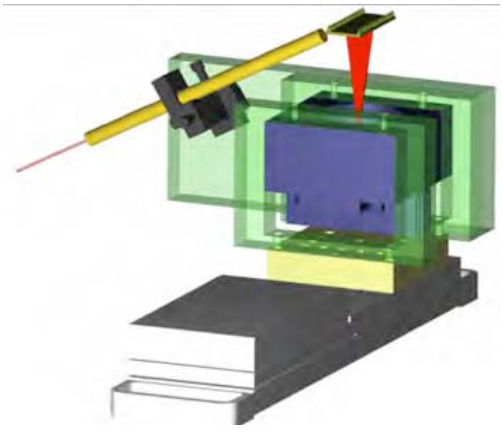

Fig. 4. Sketch of the optical system for the interrogation of the optical biochip

The instrument is directly connected to a laptop where a customised User Interface (developed by Datamed as well) allows the controls of the fluidics (Fig. 5).

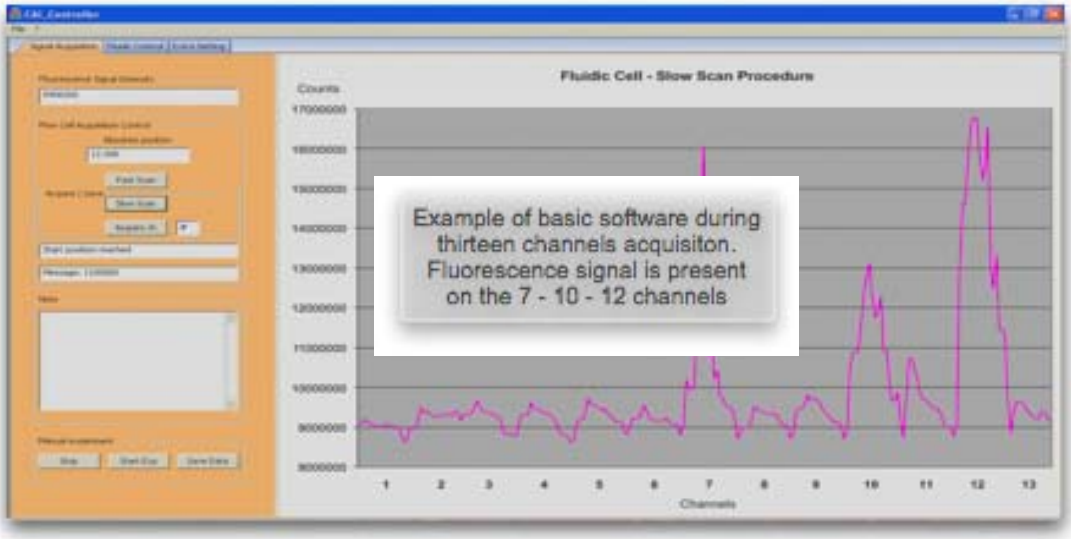

Fig. 5. Representation of the software interface

3. Chemical protocols

3.1. The immobilisation procedures

Two different approaches for the capture antibodies (Ab) immobilisation were used: simple adsorption on the PMMA surface and covalent immobilisation.

The first step consisted in the deposition of the capture antibody, clone C5 specific for CRP (C reactive protein), clone PROC1 3G3 specific for PCT (procalcitonin) and antibodies without affinity for CRP and PCT for the analysis of the unspecific binding. Different concentrations of the capture Ab were tested as detailed described in the following paragraphs. As for the simple adsorption procedure, the clone C5 and PROC1 3G3 were let flow through the channels and kept for over night interaction with the PMMA surface at 4°C. For the covalent immobilisation a functionalisation of the PMMA surface was needed. Two different functional groups were used: -COOH and NH_2 groups.

As for the carboxylic group, the channels were functionalised by depositing 0.01 mM Eudragit L100® in EtOH 95%, until the evaporation of the solvent. The two parts (cover and black bottom) of the chip were then sealed. The syringe pump of the instrument was then used for all the following steps at a flow rate of 100 μL min^{-1}. After the activation of the PMMA carboxylic groups by means of 2 mM 1-ethyl-3-[3- dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 5 mM N-hydroxysuccinimide (NHS), the capture mAb were covalently immobilised. After 5 min of washing with buffer solution, the carboxylated sites still active on the PMMA surface were inactivated with 1 M ethanolamine. After 5 min of washing with buffer solution, the surface was blocked by pumping for 15 minutes a commercially available blocking solution® and finally washed for 5 minutes with buffer solution.

The chips with the amino groups were provided already functionalised by the German company Chip & Shop. The activation of the carboxylic groups with EDC/NHS was done on the capture Ab itself. Then the same procedure described before was followed.

3.2. The sandwich assay protocol

The sandwich assays were performed pre-incubating the antigens CRP and PCT respectively with DY647-labelled mAb C7 and PROC4 6C6. Both the pre-incubation and the incubation into the channel were performed for 10 min. Different concentrations of the antigens were used in order to observe a different response of the sensor. All these steps were performed filling the channel at a flow rate of 100 µL min^{-1} and then stopping the flow.

In some chips LowCross buffer® was used to dilute the antigen, in order to reduce the not specific binding. More details are given in the following paragraph.

4. Results and discussion

Since a characterisation of the new prototype and of the PMMA-chip was necessary, different chemical treatments were performed. For more clarity, a table of some representative protocols and the related measurements are here presented. The gray columns in the tables are related to the channels not used for the assay procedures. In all the measurement graphs, the pink line is the background of the chip scanned in presence of the buffer and the blue line is the chip scanned after the sandwich assay and washed with the buffer.

1	2	3	4	5	6	7	8	9	10	11	12	13
	Cap Ab: [IOC7E1]= 100 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Preincub. Sandw. ass [CRP]=0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 500 mg/L Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 500 mg/L Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 500 mg/L Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 500 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 500 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [IOC7E1]= 500 mg/L Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L

Tab. 1. Protocol of chip NH_2b8

In table 1, the treatment done on chip NH_2b8, in which the functional groups (-NH_2) were used for the covalent immobilisation of the capture Ab, is schematically represented. Two different concentrations of the capture Ab (100 and 500 mg L^{-1}) were used and moreover a capture Ab, non specific for the CRP (IOC7E1), was immobilised.

The detection Ab used was the clone C7 labelled with DY647 (concentration: 10 mg L^{-1}). A sandwich assay with a pre-incubation of the analyte and the detection Ab was performed. Both the pre-incubation and the incubation into the channel were 10 minutes long.

The scanned chip showing the different fluorescence signal related to the different concentrations of the CRP is showed in Fig. 6.

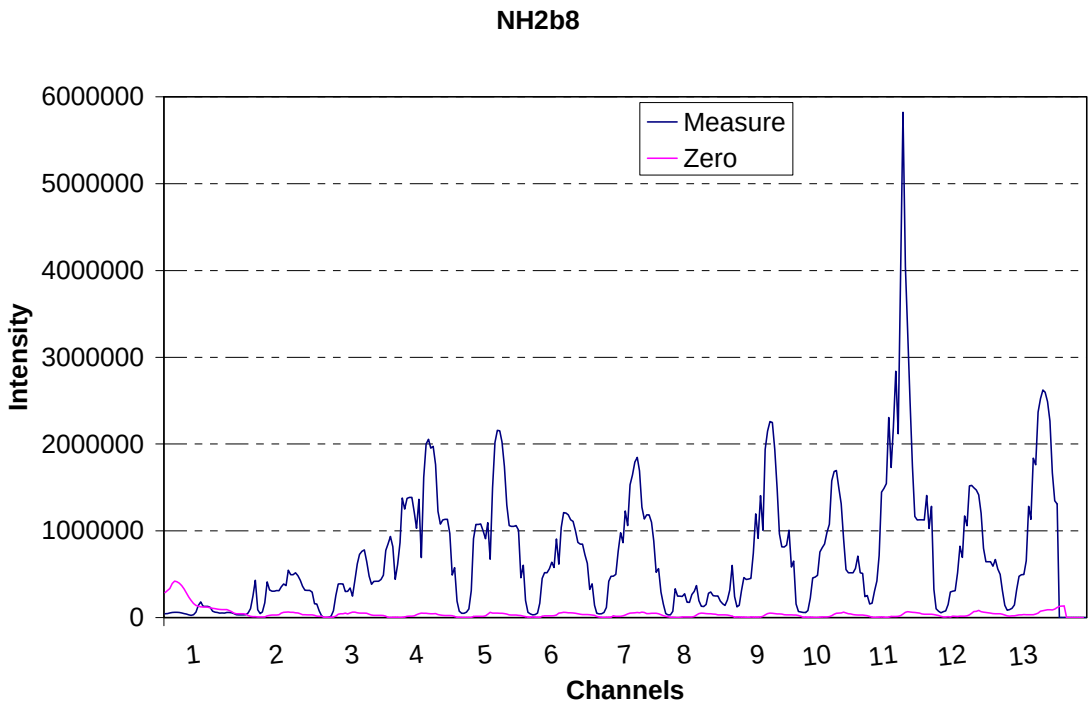

Fig. 6. 13-channel chip measured before and after the sandwich assay on NH_2b8

It is possible to observe a not perfect sealing between channels 3 and 4, in which the signal seems not well separated, moreover there is some unspecific binding on channel 13 in which the Ab IOC7E1 is immobilised.
Following the advises of Dr. Kraemer, a well-known expert in immunoassay and surface preparation, the adsorption of the capture Ab was tested, instead of the covalent immobilisation on functional groups. Table 2 illustrates the procedure followed for the preparation of chip COOHb1, characterised by COOH functional groups.

1	2	3	4	5	6	7	8	9	10	11	12	13
Cap Ab: [IOC7E1]= 100 mg/L in PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L In PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L In PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L In PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L In PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L In PBS Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [IOC7E1]= 100 mg/L in Carbon. Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Carbonate Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Carbonate Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Carbonate Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Carbonate Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 100 mg/L Carbonate Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 100 mg/L Det. Ab: [C7*]=10 mg/L	

Tab. 2. Protocol of chip COOHb1

Only one concentration of the capture Ab was let flow in the channels (100 mg L^{-1}) and two different buffers were used, PBS (40 mM phosphate buffered saline, pH 7.4) and carbonate (50 mM carbonate buffer, pH 9.6-9.8). Moreover, the Candor blocking solution (diluted 1:5) was used for the surface passivation. The results achieved with this protocol are showed in Fig. 7.

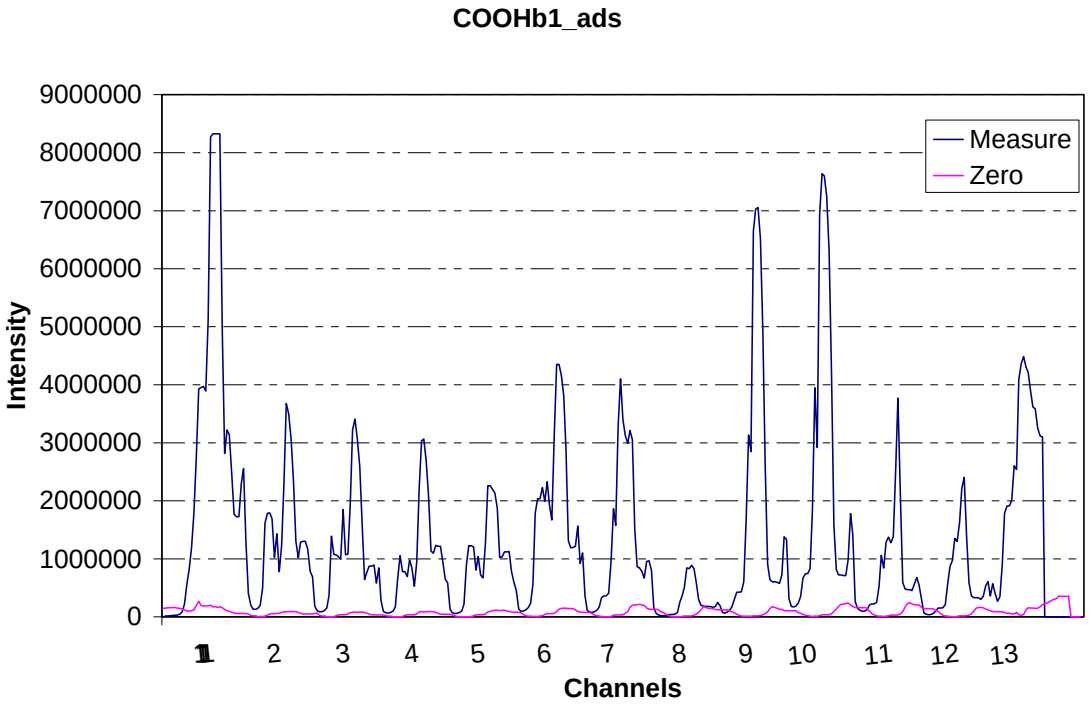

Fig. 7. 13-channel chip measured before and after the sandwich assay on COOHb1

The results were quite good in terms of fluorescence signal, but a too high unspecific binding was still present. For the third chip a lower concentration of the capture Ab (10 mg L^{-1}) was used and let interact with the surface for the whole night. Moreover the non specific capture Ab was changed in order to understand the reason of the unspecific interaction (Tab. 3).

1	2	3	4	5	6	7	8	9	10	11	12	13
	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=0.1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [AbDNT]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [AbDNT]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=0.1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	

Tab. 3. Protocol of chip COOHb2

In Fig. 8 it is possible to observe that the unspecific binding was still not clear (channels 6-7) and a possible Hook's effect was observed when the concentration of CRP was too high (channel 12 with CRP 100 mg L^{-1}). The so called Hook's effect is observed at a high concentration of the analyte, which means a decrease of the fluorescence signal with a further increase of the CRP concentration. This effect can be due to an inefficient washing

step after the incubation with the antigen or to heterogeneity of the assay. In the first case, the incomplete removal of the unbound analyte in the washing step can lead to its removal during the second incubation step, with the detection antibody, and to its consequent interaction with the free detection antibody. This would imply a decrease in the number of the detection antibody available for the binding to the solid phase and consequently a decrease in the detected fluorescence signal. In the second case, it is necessary to consider that the capture antibodies after the immobilisation do not behave exactly in the same way as they do in solution; therefore, slightly different variations in the binding constants can be expected in the immobilised antibodies. On the basis of these considerations, a different behaviour of the immunochemical reaction can be expected in the presence of low and high concentrations of the investigated analyte. When the concentration of the analyte is low, only the antibodies characterised by the highest value of the affinity constant are involved in the immunoassay. In the presence of high concentration of analyte, also the antibodies characterised by lower binding constants will interact with the analyte. In the case of the interaction of the analyte with the immobilised capture antibodies, there is the formation of weaker immunocomplexes which can dissociate during the second incubation step of the sandwich assay. The dissociated analyte will interact with the free labelled detection antibody. This interaction will imply a decrease of the labelled detection antibodies which take part in the formation of the sandwich on the sensing layer and will lead to a decrease of the detected signal. This effect can be eliminated by increasing the concentration of the detection antibody.

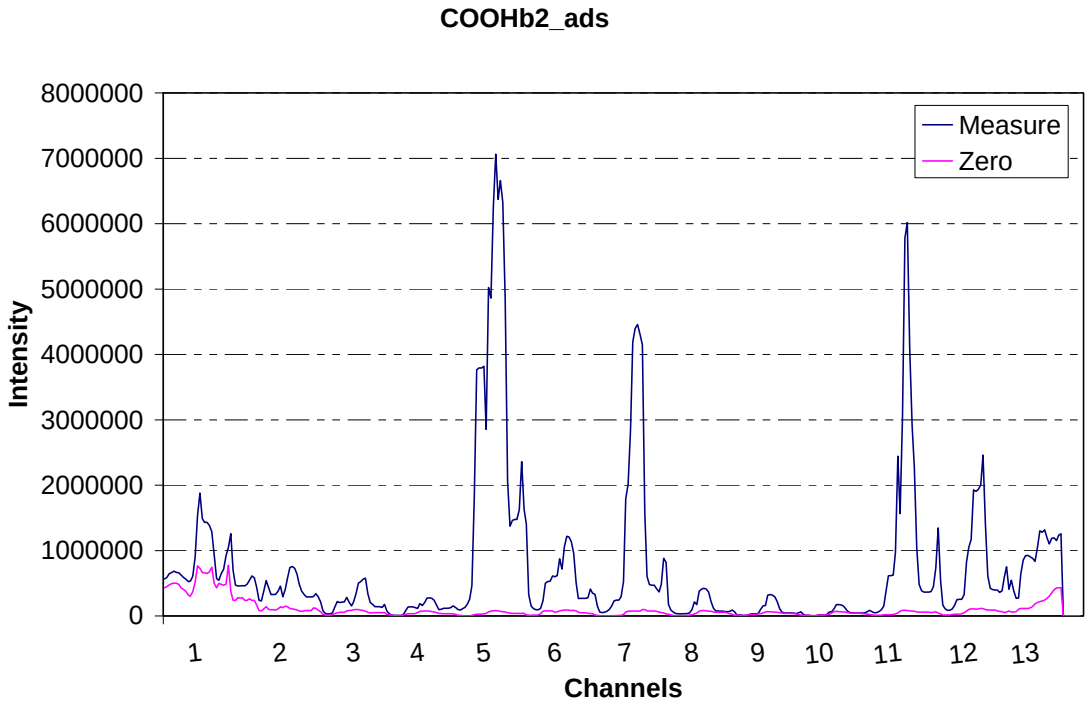

Fig. 8. 13-channel chip measured before and after the sandwich assay on COOHb2

The same protocol was then tested on a chip functionalised with the NH_2 groups, still with the over night capture Ab deposition (Tab.4).

1	2	3	4	5	6	7	8	9	10	11	12	13
Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0.1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [AbTNT]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [AbTNT]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=0.1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 1:5 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	

Tab. 4. Protocol of chip NH_2b9

In Fig. 9 the results obtained with the same protocol applied on the surface functionalised with the amino-groups, are shown and it is interesting to observe that the trend of the results is the same: high unspecific binding (channel 7) when the concentration of the analyte is 100 mg L^{-1} and a possible little Hook's effect (channel 12).

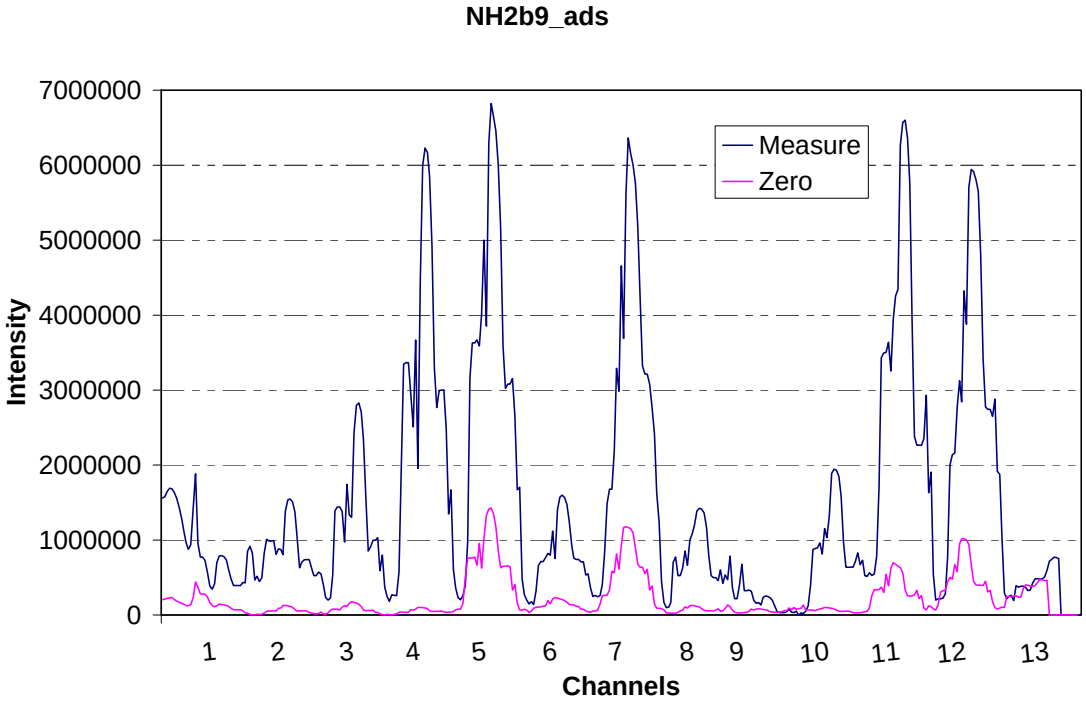

Fig. 9. 13-channel chip measured before and after the sandwich assay on NH_2b9

On the next chip (Tab. 5) a further change was done: the non-diluted blocking buffer was used for the passivation of the surface. In this chip not only the CRP at different concentrations was tested but also the PCT, using as capture Ab the specific clone PROC1 3G3 and PROC4 6C6 labelled with DY647 (2 mg L^{-1}), as detection Ab. The test for the specificity was done incubating the CRP with the capture Ab for PCT and vice versa the PCT with the capture Ab for CRP.

1	2	3	4	5	6	7	8	9	10	11	12	13
Cap Ab: [C5]= 10mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]= 0 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]= 0.1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=10 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=1 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=10 mg/L Det. Ab: [6C6*]=2 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=100 mg/L Det. Ab: [C7*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]= 0 mg/L Det. Ab: [6C6*]=2 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=0.01 mg/L Det. Ab: [6C6*]=2 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=0.1 mg/L Det. Ab: [6C6*]=2 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=1 mg/L Det. Ab: [6C6*]=2 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=10 mg/L Det. Ab: [6C6*]=2 mg/L	

Tab. 5. Protocol of chip NH_2b10

In Fig. 10 the fluorescent signal obtained from chip NH_2b10 are showed.

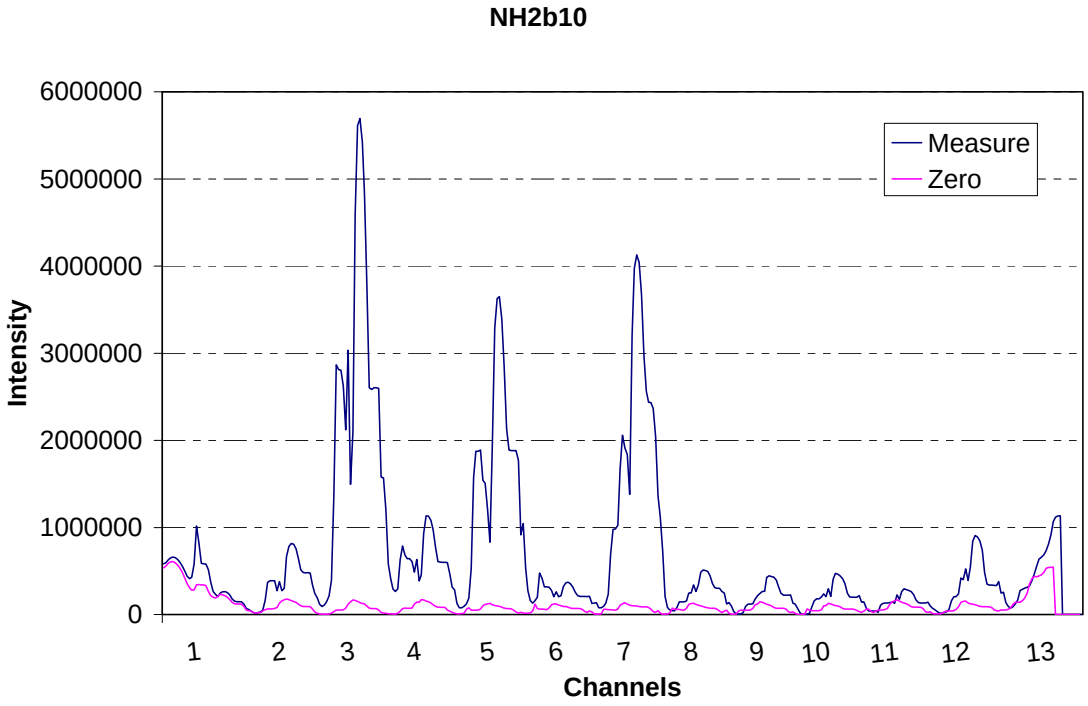

Fig. 10. 13-channel chip measured before and after the sandwich assay on NH_2b10

The unspecific binding for CRP was not solved yet, since on channel 7 the signal was still high and some more attempts were needed for PCT, since not detectable signals were achieved.
On the next chip (Tab. 6), the low-cross binding solution (LCB diluted 1:5) was added to the pre-incubation step of the CRP and the detection Ab of the PCT was increased to 10 mg L^{-1}.

1	2	3	4	5	6	7	8	9	10	11	12	13
Cap Ab: [C5]= 10mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]= 0 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]= 0.1 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=1 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=10 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=100 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=100 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [C5]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=10 mg/L Det. Ab: [6C6*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [CRP]=100 mg/L LCB 1:5 Det. Ab: [C7*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=0 mg/L Det. Ab: [6C6*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=0.01 mg/L Det. Ab: [6C6*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=0.1 mg/L Det. Ab: [6C6*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=1 mg/L Det. Ab: [6C6*]=10 mg/L	Cap Ab: [3G3]= 10 mg/L Candor Bloking solution 15 min Preincub. Sandw. ass [PCT]=10 mg/L Det. Ab: [6C6*]=10 mg/L

Tab. 6. Protocol of chip NH_2b11

In Fig. 11 the fluorescent signal obtained from chip NH_2b11 are showed. Quite good results were obtained since from channel 8 it is possible to observe that the unspecific interaction of CRP was almost removed, but unfortunately some problems on the concentration distribution needs still to be investigated. However the results with PCT were quite satisfactory since an increase of the signal detected following the concentration was observed.

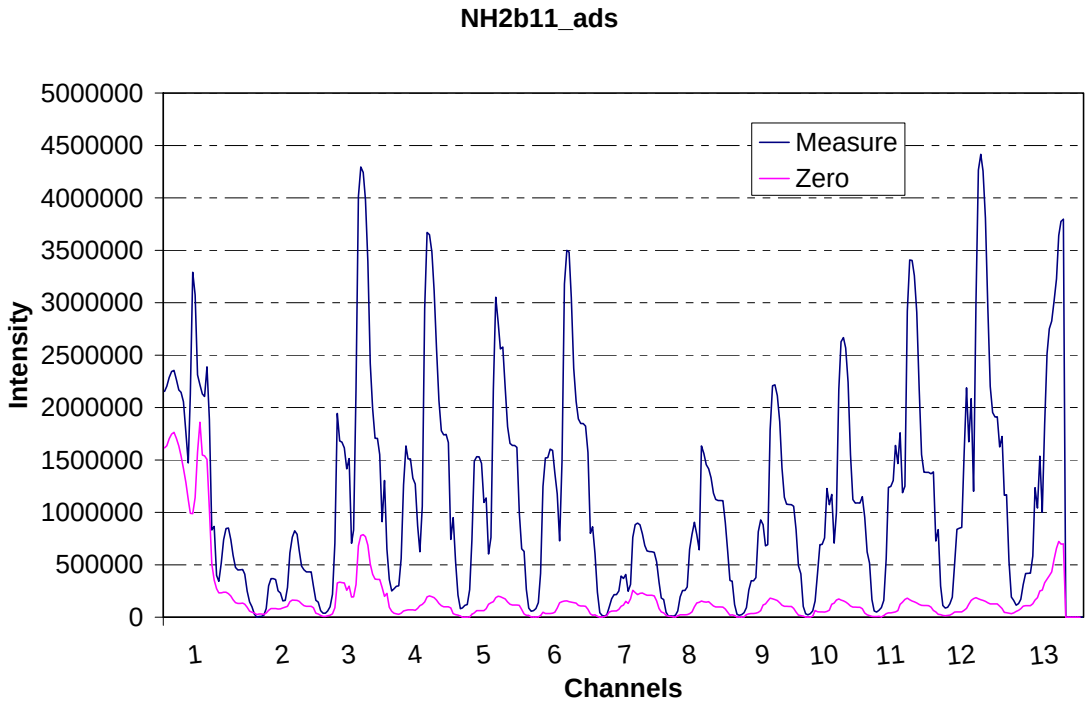

Fig. 11. 13-channel chip measured before and after the sandwich assay on NH_2b11

At the moment, the measurements performed with the prototype developed within the project were only partly confirming the results obtained in the previous years during the development of the chemical protocols and the set-up on the optical bench. The not perfect correlation between the concentration of the analyte and the fluorescent signal acquired were probably due to the not perfect water-tightness of the microchannels in the chip.

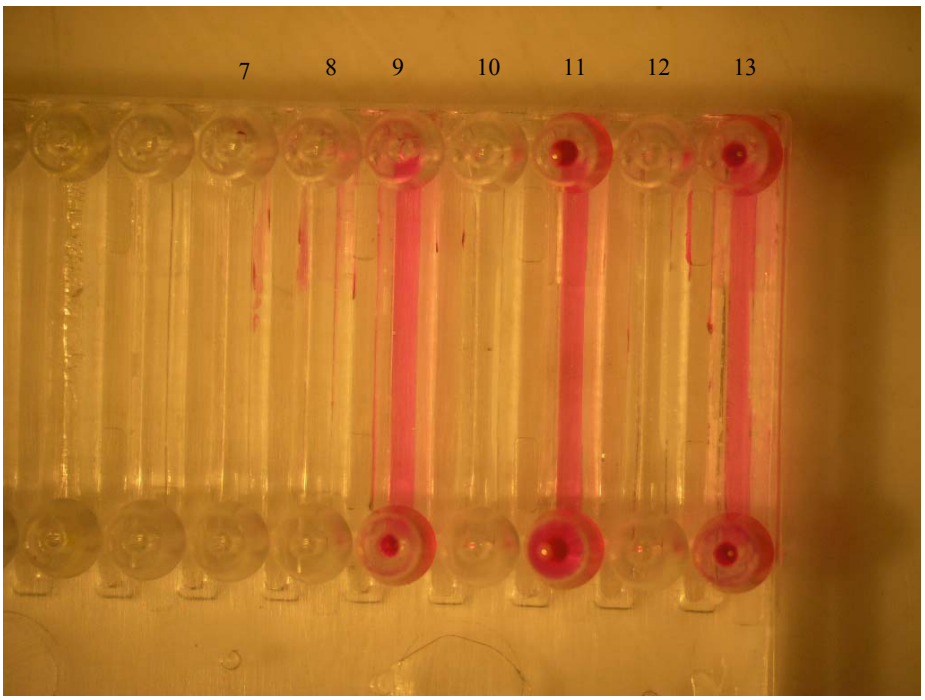

Fig. 12. Solution of Rhodamine B in water deposited in channels 9-11-13

In particular, a solution of Rhodamine B in water was injected into a fully transparent chip by a syringe. As it can be observed in Fig. 12, channels 9-11-13 were filled-in with the solution, but rhodamine traces are visible also in channels 7-8-12.
Then some chips with the black bottom were tested. Due to the difficulties to observe small leakage between channels when the bottom is black, an attempt with milk was done. With this method it is not possible to see traces of the white solution in the contiguous channels, but was interesting to see that the milk was completely going between channels 1 and 2 (Fig. 13).

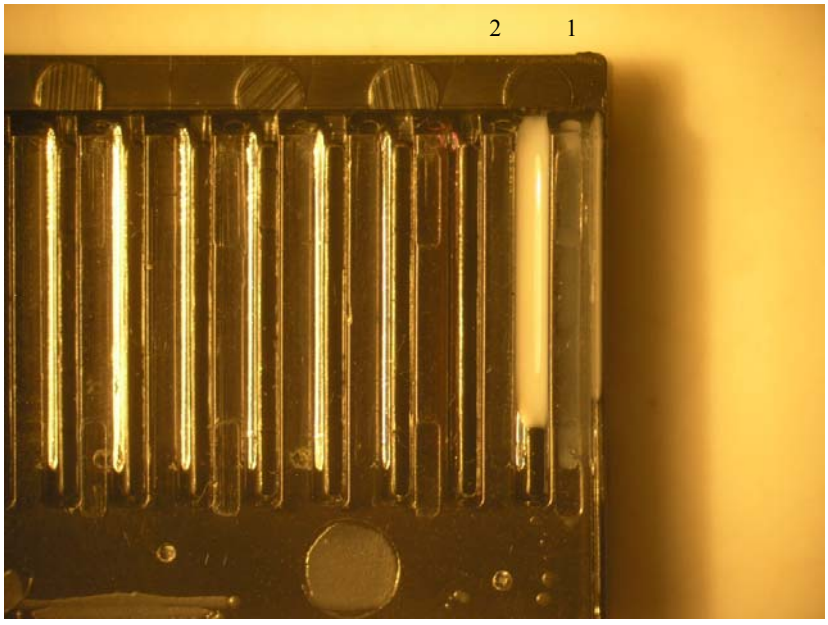

Fig. 13. Milk leakage between channels 1 and 2

Then a solution of fluorescein in water was tested and a blue led was used to excite the fluorescence. As we expected, fortunately, not all the channels had the leakage problems as it can be observed in Fig. 14.

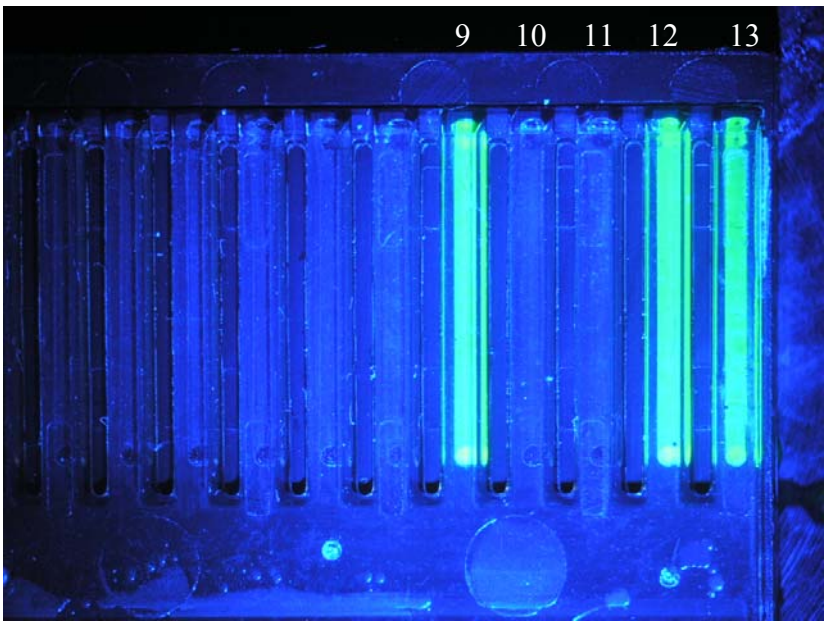

Fig. 14. Solution of fluorescein in water deposited in channels 9-12-13

But since some of them are subject to leakage (Fig. 15), it is obviously possible to have confusing results because of a cross-talk among the channels.

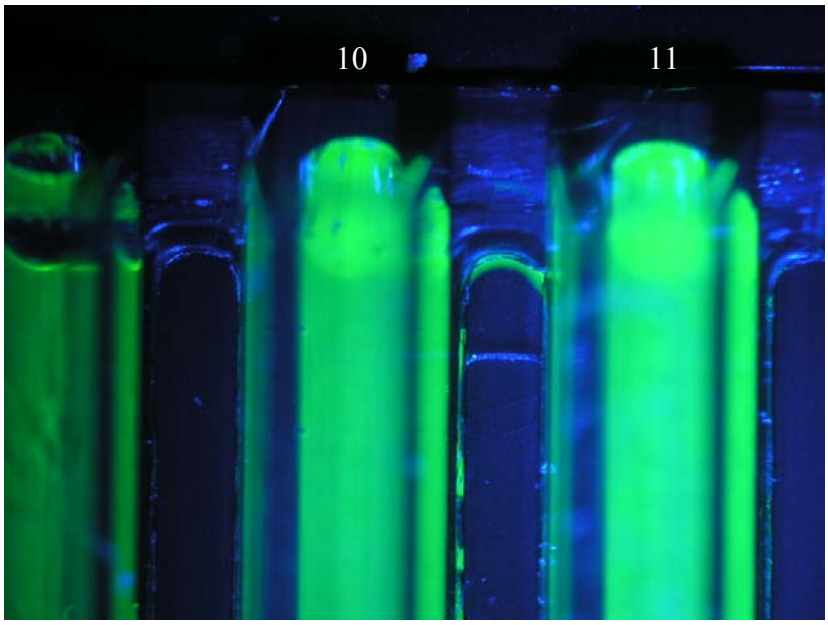

Fig. 15. Evidence of leakage between channels 10 and 11

At the end of the period spent at Helmholtz Zentrum München, since the strict collaboration with Dr. Krämer within the CARE-MAN project, further immunoassays

with the prototype were considered necessary and then scheduled. Because of that, training on the software, on how to handle the instrument and on the chemical protocols applied, was carried out for the technicians working with Dr. Kraemer.

5. Conclusions

Measurements on a newly designed multi-channel array for sepsis markers investigation were performed. Not only the developed prototype was tested, but also the chemical protocols and the leakage problems were faced and partly solved. Considering the promising results obtained with the POCT prototype, further investigations resulted necessary and a training of technicians working in the host laboratory was performed.