Using ULS24 as a read-out sensor for chemiluminescence immunoassay and DNA hybridization assay

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Summary

In this report, we describe the application of ULS24 in detecting chemiluminescence emision in immunnoassay and DNA hybridization chemistry. 4 different test set ups are descibed here,ranging from standard plate based setup to microfluidic configuration with ULS24 coupled to the fluidic chip in a lensless fashion. We discuss the potential limit of detection (LOD), dynamic range, multiplex potential etc. with each set up. Lastly, we report that with microfluidic setup, it is possible for ULS24 to help achieve a sensitivity of several pico gram per mL sensitivity with only 10 minute of so incubation time in chemiluminescence immunoassay. This could potentially set stage for a wide variety of point of care clinical testing applications.

Introduction

Immunoassay is a widely used method for detecting the presence and quantifying a macromolecule in a solution. This is accomplished through the use of an antibody molecule. The macromolecule, or often referred as "analyte", that is detected by the immunoassay is most often a protein molecule. Analytes in biological liquids such as serum or urine are frequently measured using immunoassays for medical and research purposes. One of the most popular and effective immunoassay methods is the enzyme-linked immunosorbent assays, or ELISA

Chemiluminescent immunoassays are variations of the standard ELISA. An enzyme converts a substrate to a reaction product that emits photons of light instead of developing a visible color.

Chemiluminescence is light produced by a chemical reaction. The chemiluminescent substance is excited by the oxidation and catalysis forming intermediates. When the excited intermediates return back to their stable ground state, a photon is released, which is detected by the luminescent signal instrument.

Chemiluminescent assays, in particular certain enhanced chemiluminescent assays, are very sensitive and have a wide dynamic range. It is believed that chemiluminescence is the most sensitive detection method currently in use due to the ability of signal multiplication and amplification. Chemiluminescent reactions are measured in relative light units (RLU) that are typically proportionate to the amount of analyte present in a sample.

In order to measure light emission from chemiluminescence, photon multiplier tube (PMT) devices are typically employed in the read-out instrument. PMTs are very sensitive light detection devices. However, PMT, along with the necessary electronic circuits to make PMT work, is very bulky and expensive. As such, chemiluminescence read-out instruments are also bulky and expensive.

Here we introduce the use of highly integrated and ultra-low-light sensitive CMOS bio-imager sensor chip to detect chemiluminescence emission from chemiluminescence immunoassay. CMOS bio-imager device described here is a true image sensor. It can potentially be used to detect the emission of chemiluminescence emission from multiple reaction sites at the same time.

Methods and Results

Experiment 1: Single target reading

Materials and method

Chemiluminescence immunoassay material: Akaline Phosphadase (AP) (part number: ALPI12G), from BBI Solutions (info@bbisolutions.com).

Luminol Substrate (part number: SR2001), Jiangsu ZECEN Biotechnology Co., Ltd, PR. China.

Plate: White opaque 96 well microtiter plate, from Thermo Scientific.

Sensor: Anitoa ULS24 Ultra-low-light CMOS bio-imager. ULS24 Solution Kit 1-Channel System.

Lens: a F1.0, 16mm CS mount lens (part number: GMF1610CIR). The aperture of the lens is also 16mm. Supplier: Yian Optics Co. Ltd, PR. China (http://www.yagx.net).

Software and settings: Anitoa ULVision software. The integration time is set to 5s to 60s. Resolution is set to 12x12. Binning mode pattern is 0xF (all 4 sensors within a big pixel are turned on).

Ambient temperature: 15-25 degree C.

Below picture shows the setup of the experiment. The CMOS sensor is mounted above the plate and it images the reaction tube from top. The enzyme mix, when activated, emits blue light centered at ~460nm wavelength. We do not use any filters for this setup.

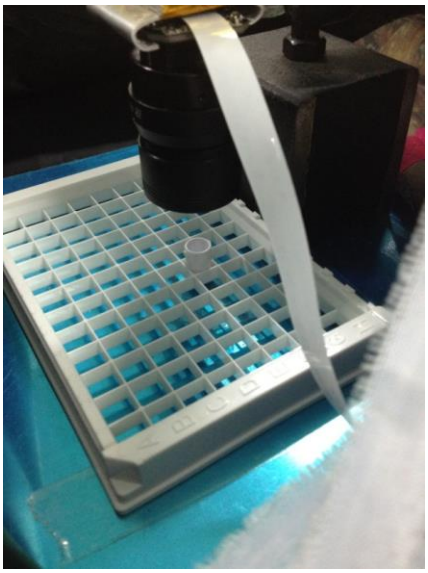


Figure 1, Chemiluminescence measurement setup

Procedure

First of all, the stock AP enzyme master mix is diluted 2000 times. This is done by taking 0.5ul of 100ug/mL master mix; add it to 1000ul Tris Buffer (ph8.0) in a reaction tube. The tube is vortexes well to ensure a good mix. Then the tube is allowed to set for 15min. We then take 200ul diluted mix and put into 800ul Tris Buffer (pH8.0), shake to mix, to further achieve another 5x dilution.

Use same method above to create 5 different concentration of reaction mixture: 0.05, 0.01, 0. 002, 0.0004, 0.00008ug/mL).

After all set up is complete, fetch 1ul of enzyme mix, add to tubes that contain 50ul of luminal substrate. Start with lower concentration samples first and finish with the highest concentration sample. This is because the light attenuation is faster with higher concentration samples.

Figure 2 is a sample image taken by the sensor from the top of the test tube. As can be seen, the whole tube is imaged in the 12x12 pixel array area. To calculate the read out, we just simply add the output from all 144 pixels.

We also image the tube with luminol substrate, but with no enzyme added. This serves as negative control. When we calculate the light output from the sample images, we always substract negative control image from the image taken from the sample.

Below is the read out light level from samples of different enzyme concentration:

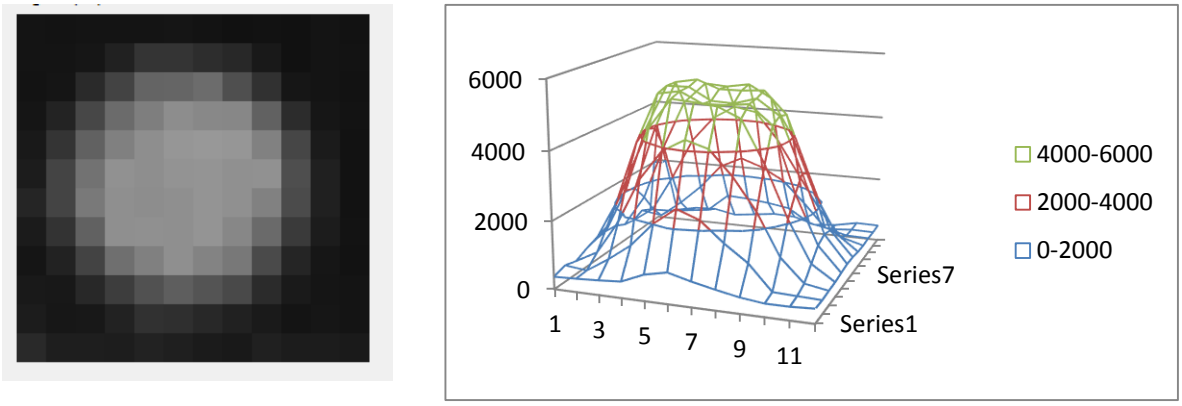


Figure 2, Sample image and its 3D histogram, from the image sensor that is performing the read out

Enzyme concentration µg/mL	Light signal output
0.05	1611321.5
0.01	306904.0
0.002	66950.4
0.0004	27796.9
0.00008	8958.3

Table 1, Chemiluminescence light readout vs. enzyme concentration

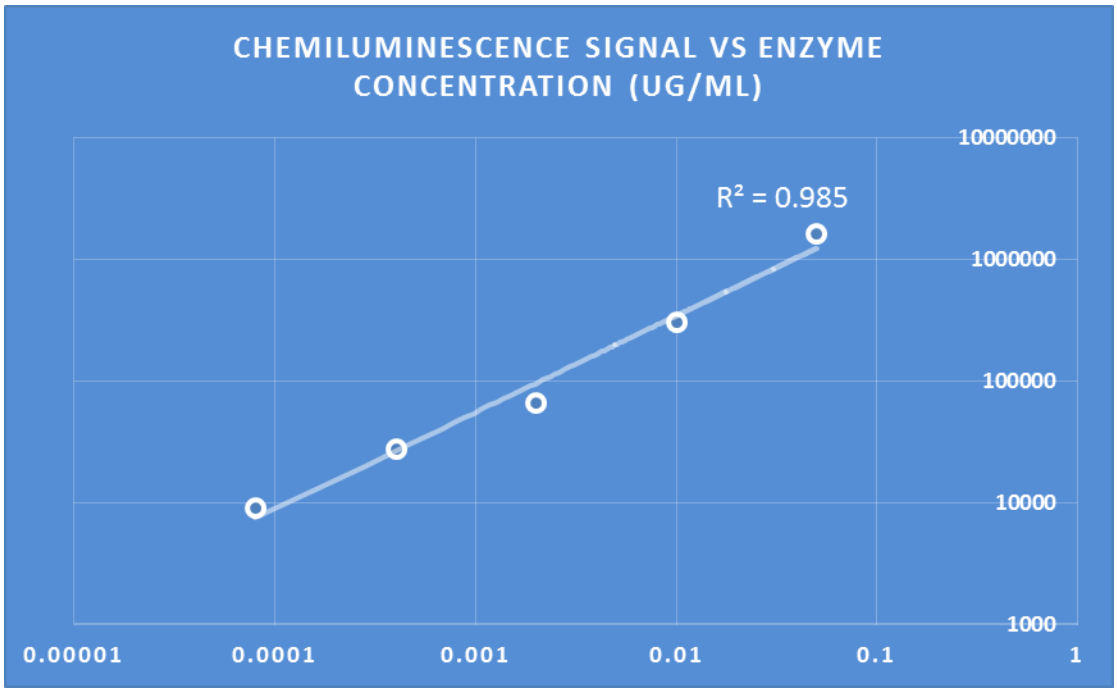


Chart 1, Chemiluminescence light readout vs. enzyme concentration in logarithm scale, fitted with a linear interpolation curve.

Experiment 2: Multiplexed reading

With some small adjustment of the optics setting, namely object distance and lens focus, we were able to test multiple samples with one single image shot. Below are the procedures and results of this test.

First we imaged 4 empty test tube in ambient light with 10-20ms integration time. We did both 24x24 and 12x12 shots, while adjust the lens focus.

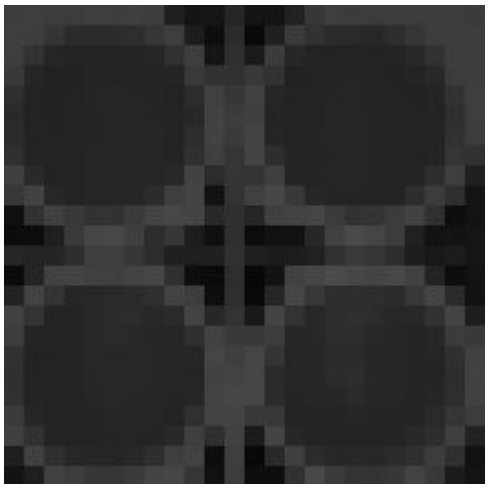


Figure 3a Image of 4 empty test tube in ambient light, 24x24 resolution mode

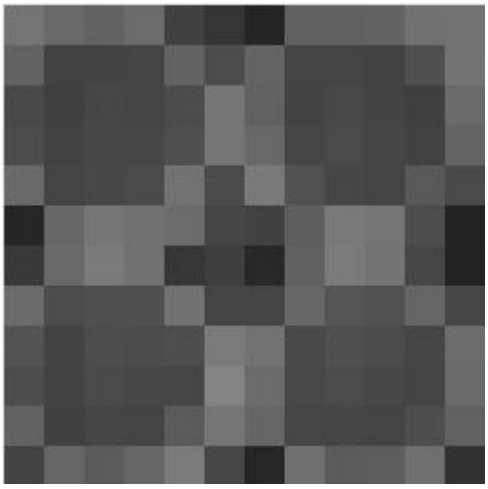


Figure 3b Image of 4 empty test tube in ambient light, 24x24 resolution mode

As can be seen in the screenshot, even in 12x12 mode, we can reliably distinguish the 4 test tubes.

We then placed the system in dark enclosure and imaged the tubes with longer integration time. We added ALP enzyme with luminol substrate in the up-left corner. The other 3 tunes contain only luminol substrate, but no enzyme. We performs a series of test and the result is shown below.

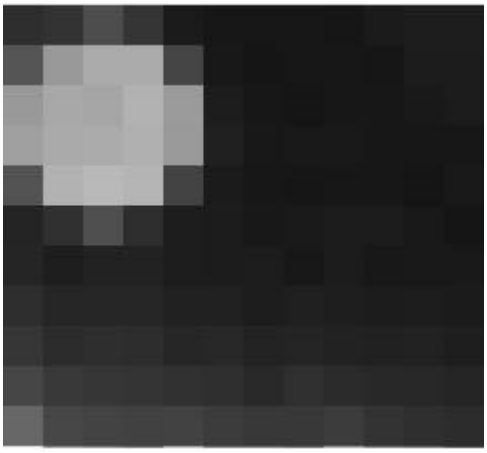


Figure 4. Screen shot of the image with upper left corner test tube filled with ALP enzyme and luminol substrate, while the other 3 tubes contain only luminol substrate, with no enzyme. The enzyme concentration from this screen shot is 0.002ug/ml.

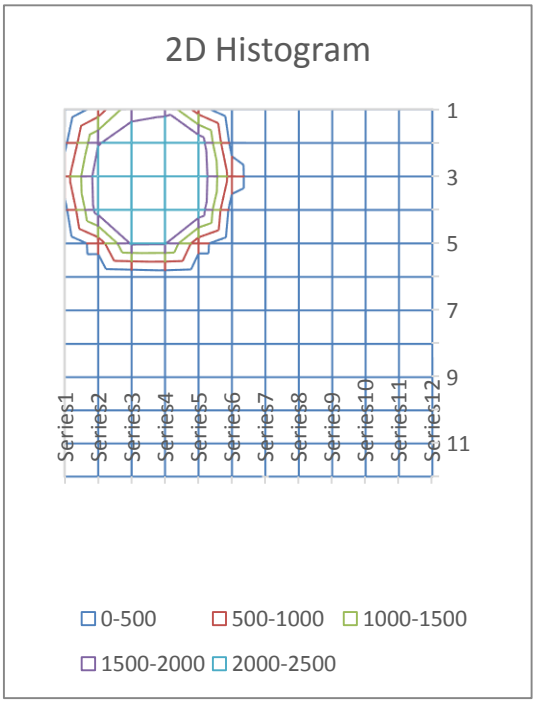


Figure 5a 2D histogram of the test described above

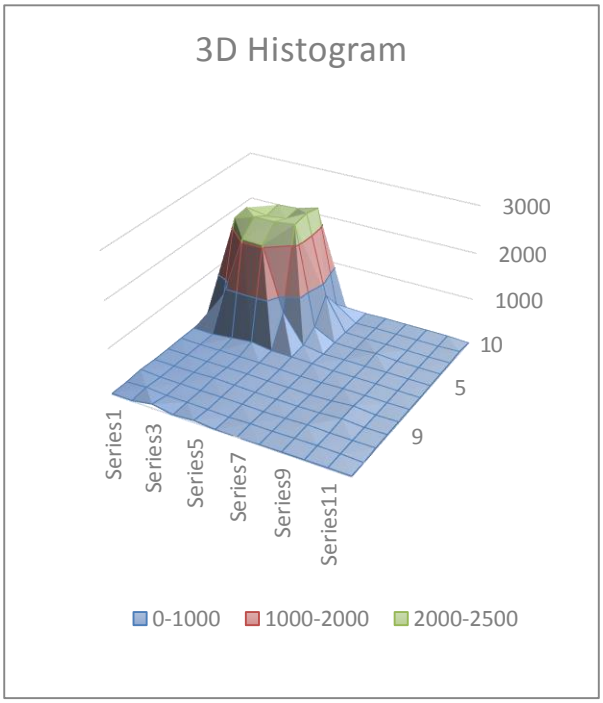


Figure 5b 3D histogram of the test described above

Note that when the histogram is produced, we performed dark subtraction to reduce background noise.

We performed the 3rd, 4th and 5th latter concentrations (0.00008ug/ml, 0.0004ug/ml, and 0.002ug/ml)

Enzyme concentration (µg/mL)	Chemiluminescence Readout
0.002	54244.93
0.0004	19897.19
0.00008	4181.16
0	81.23

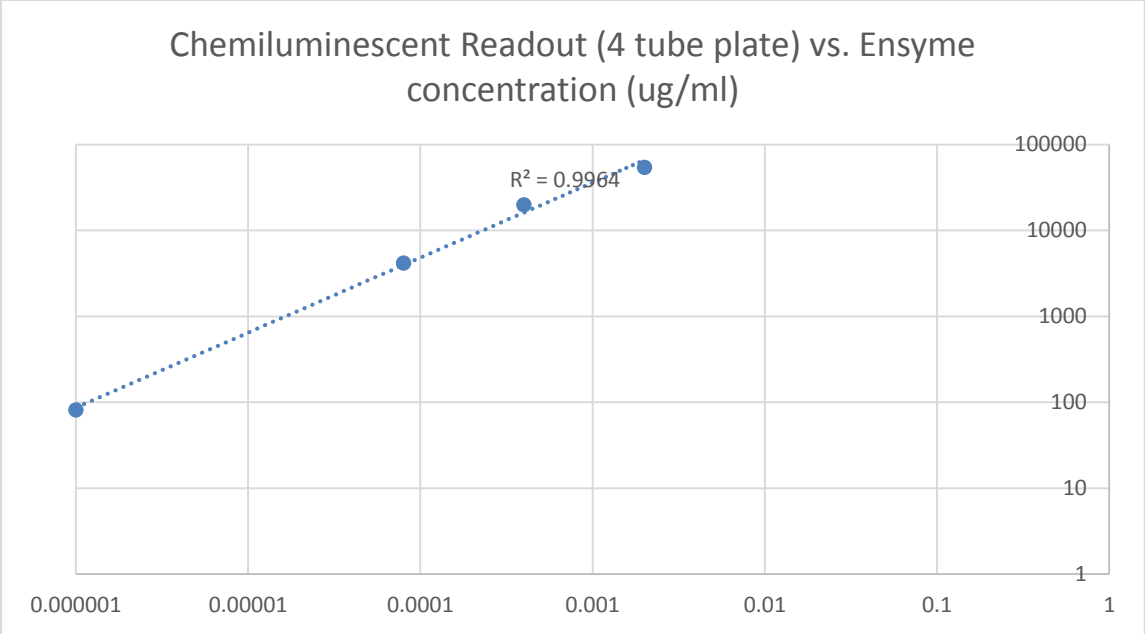


Chart 2, Chemiluminescence readout vs. enzyme concentration in logarithm scale, in a 4 tube plate format

Experiment 3: DNA hybridization in a configuration that directly couple CMOS Bio-optical sensor to the assay

It is also possible to directly couple Anitoa CMOS Bio-optical sensor to the chmeluminescence assay. This method has the highest light collection efficacy and the advantage of simplicity. Below is an example of ULS24 interfacing with a microfluidic chip.

With this method, it is possible to detect ng/ML level of chemiluminescence analyte with shorter integration time, for example 1-5 second of integration time.

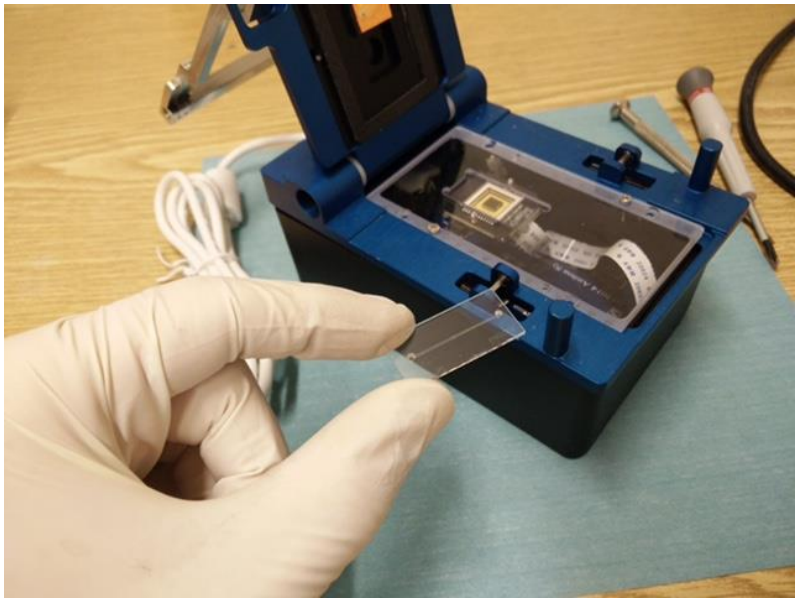


Figure 7 ULS24 sensor directly coupled to a microfluidic chip running beads based chemiluminescence immunoassay (Image provided by Micronit BV).

In a separate experiment (performed also in collaboration with Micronit BV), the ULS24 CMOS biosensor is used in conjunction with a microfluidic chip in a lens-less configuration (Figure 8a,b) for beads-based DNA hybridization. The capture DNA probes are attached to magnetic beads, and the detection probes were coupled with HRP enzyme. As the target DNA in a sample (e.g. PCR product) hybridize with the probes, they are trapped at the detection point in the microfluidic chip due to a small magnet.

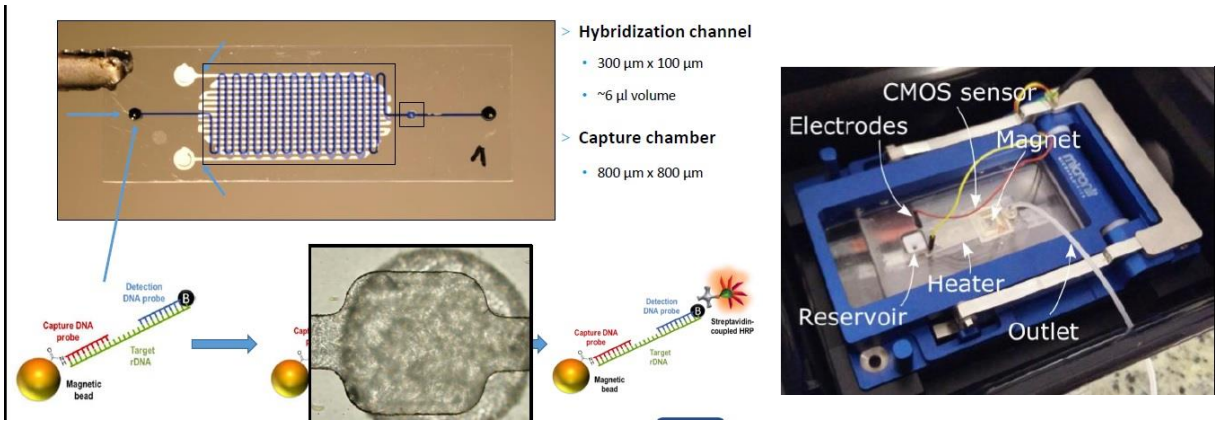


Figure 8a, b Microfluidic and beads based DNA hybridization set up, where the ULS24 CMOS biosensor is tightly coupled to the microfluidic chip in a lens-less configuration to detect chemiluminescence

To detect the hybridized target DNA, a luminol substrate is passed through the channel. As the HRP-conjugated probes react with the substrate, chemiluminescence emission is generated and detected (Figure 8c).

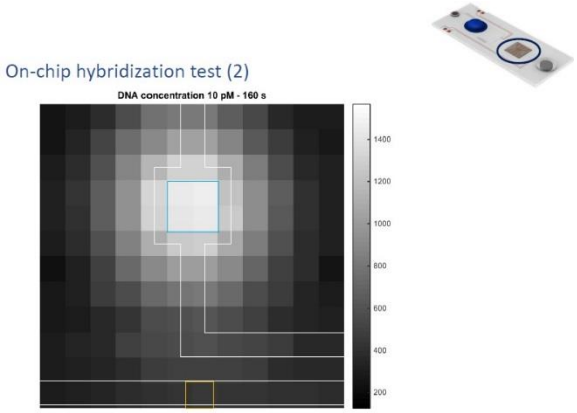
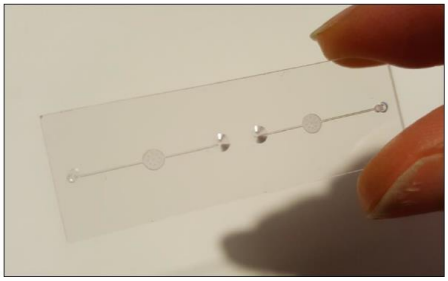


Figure 8c Chemiluminescence image captured by ULS24 CMOS Biosensor

Experiment 4: Chemiluminescence immunnoassay on a chip

In this experiment, a complete chemiluminescence immunoassay test is carried out, using a set of synthetic antibody/antigen compounds (Synthetic ICAM-1). The reaction is carried out in a simple microfluidic chip illustrated in figure 9 (provided by Micronit BV). This design of this chip consists of a reaction chamber, an inlet with fluidic reservoir and an outlet. Actuation is achieved by creating a vacuum at the outlet, for example, using a syringe pump.



- High transparent thermoplastics (COC);
- Embedded open reservoirs V_R = 5 µl;
- Capture chambers with V_C = 0.75 µL

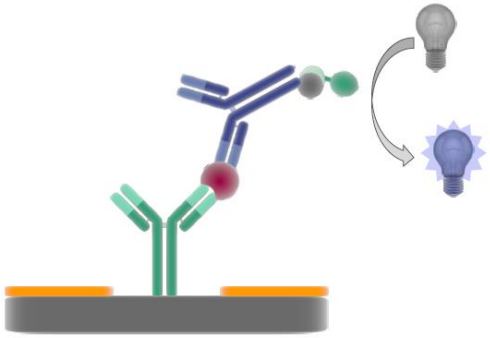


Figure 9 a. Microfluidic chip used in the experiment. b. Chemiluminescence ELISA concept

Similar to the DNA hybridization experient described above, the ULS24 sensor chip is coupled to the fluidic chip directly, in a lensless fashion (Figure 10). The ULS24 settings are listed below:

- High gain mode
- 12X12 resolution
- Binning pattern 0xF (4-bin mode selected)
- Integration time in the range of 1s to 20s

The light output is calculated by average the pixel readings after the dark background image is subtracted.

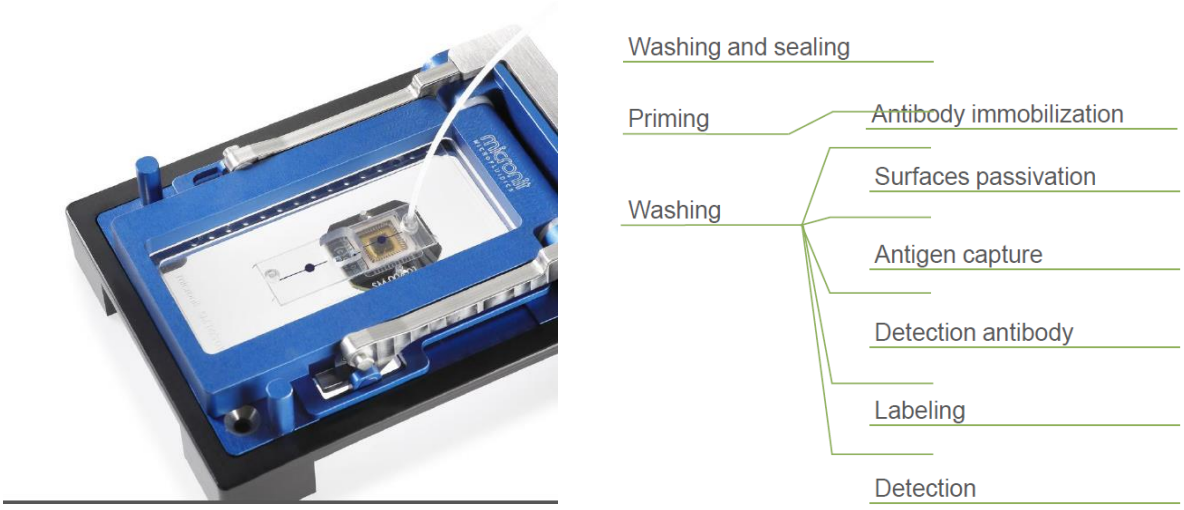


Figure 10. a. Lensless coupling of ULS24 to fluidic chip. b. Steps of the chemistry protocol

Several steps are performed to realize the protocol (Figure 10b). The first step is the chip preparation: the capture antibody is immobilized to the chamber. After this step, the chamber is sealed such that only access allowed is through inlet and outlet channels.

The actual test consists of the following step, with wash steps performed in between

1. Antigen capture. This is the incubation period. With the design described here, due to small capture chamber volume (0.75uL) and large surface to volume ratio, only ~10 minutes incubation time is required to achieve good result.
2. Detection antibody: detection antibody is added and captured.
3. Labeling: HRP enzyme is instroduced to the chamber to label the detection antibody
4. Detection: Luminol substrate is introduced to the chamber while chemiluminescence detection is performed in real time (Kinetic detection) with ULS24 sensor.

Needless to say that the steps above, especially step 4 is performed in a dark environment. We also control the temperature of the reaction chip to a set value ensure good and repeatible reactions.

Figure 11 shows the detected chemiluminescence emission as function of time and analyte concentration. As can be seen, the chemiluminescence intencity rises as the luminol substrate starts to flow through the capture/detection chamber. It then plateaus when the liminol solution achieves a steady flow. When the flow stops, the emission starts to decay. Interestingly, as the analyte concentration increases, the rate decay of signal increases too due to faster consumption of luminal substances left in the chamber.

With this method, we were able to achieve a detection threshold (LOD) of 5 pg/mL of analyte concentration, and CV of < 5%. This suggest that the combination of ULS24 CMOS bio-optical sensor and microfluidic chips can give rise to a high performance, yet compact and low cost chemiluminescence detection platform.

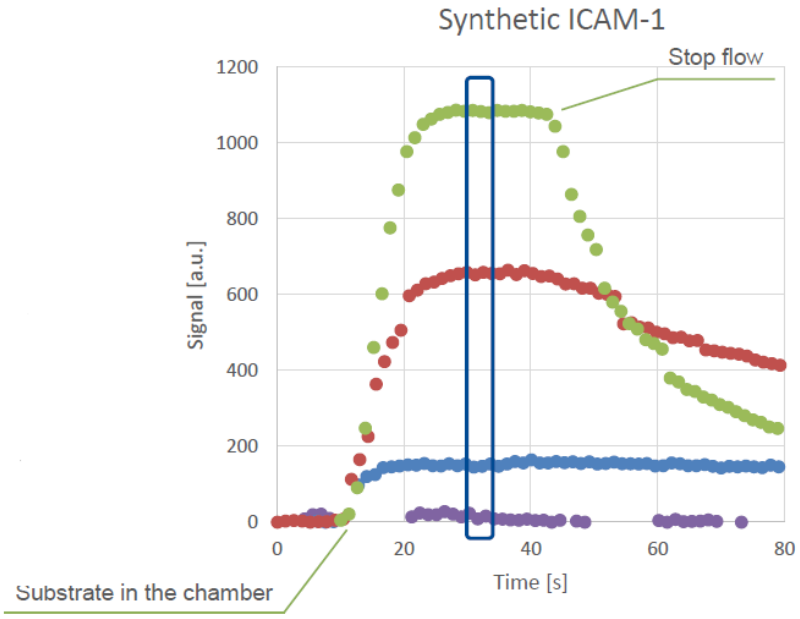


Figure 11 Chemiluminescence readout from the experiment