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C. Pieralli, C. Attaoui, W. Boireau, B. Wacogne. Low cost optical detection of DNA hybridization on biochips. *Laser Physics*, 2009, 19 (2), pp.259-264. 10.1134/S1054660X09020182 . hal-00493041

HAL Id: hal-00493041

<https://hal.science/hal-00493041>

Submitted on 14 Apr 2021

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Low Cost Optical Detection of DNA Hybridization on Biochips¹

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Abstract—The detection of DNA hybridization can be either complicated or require expensive devices. Some methods use a fluorescence signal to investigate hybridization. Here, we present an optical probe based on optical fibers both for illumination and for fluorescence collection. The detection is made with a microspectrophotometer and the signal is then treated with a PC. We also developed the DNA biochips (glass plate with a gold layer functionalized with target DNA) as well as a dedicated microtank which maintains a constant flow of probe DNA over the target one. Finally, the detection of hybridization with this simple and versatile system is presented.

1. INTRODUCTION

The DNA hybridization is a growing technique for recognizing the type of DNA strands. It consists in combining two different DNA strands (called probe and target) either with ELISA techniques [1] or by using biochips [2]. The latter is nowadays widely used because of its compactness and easy-to-use. There are various methods for hybridizing DNA: PCR like reaction [3] or deposition of thin DNA target layers [4] followed by a contact with the probe DNA. The first one deals with the use of an enzyme called DNA polymerase which combines the two DNA strands. Hydrogen bonds are therefore created between the two strands, the strength of these bonds depending on the affinity of the DNA strands. If the DNA hybridized, it resists to a heating. If not, the DNA is cut and the two strands are separated. With biochips, the target DNA is deposited onto a gold surface by creating covalent bonds. The probe DNA is then placed either with a drop or a liquid flow which make it pass on the target DNA.

In fact, the challenge is to detect this hybridization. Mainly three ways of detection are used:

- Electrochemical sensors where the hybridization is detected with an increase of an electrical current due to a redox indicator, the sensitivity being in this case rather good but it needs a heavy chemistry as well as micro-electrodes [5].

- Measurement of the weight of the DNA by using a Quartz Crystal Microbalance (QCM), always rather complicated to use, delicate to handle [6] and more-

over, it is difficult to envisage a parallelization of the detection.

- Fluorescence techniques now widely used.

Here, we present a simple optical method which permits a detection of hybridization by using fluorescence on a gold biochip. First, we will detail the optical experimental setup and its use with lipidic vesicles to estimate the characteristics of fluorescence collection. Then we will describe the elaboration of the DNA biochip dedicated to hybridization and in the next section the device specially devoted to the biochip as well as the fluorescence results. We will finally conclude by giving some perspectives with dedicated microsystems. This multidisciplinary work has been led in strong collaboration between biochemists of the Burgundy-Franche-Comté “CLinical Innovation Proteomic Platform” and physicists of the Optics Department of FEMTO-ST.

2. EXPERIMENTAL SETUP AND CHARACTERIZATION

Usually, the detection of fluorescence is made with confocal microscopy [7] or alternative methods [8, 9], mainly based on optical fibers. The first one, as it will be shown further, is the most spatially resolving but suffers from a high cost. Many works have been conducted with optical fibers [10, 11]. Most of them could be complex to implement or request a scientist for interpretation.

We propose a simplest way (Fig. 1) than the one presented in [11] consisting of two optical fibers (core: 400 μm , cladding: 500 μm , NA: 0.22), one being the emitter, the other the collector. A laser diode (P: 15 mW,

¹ The article is published in the original.

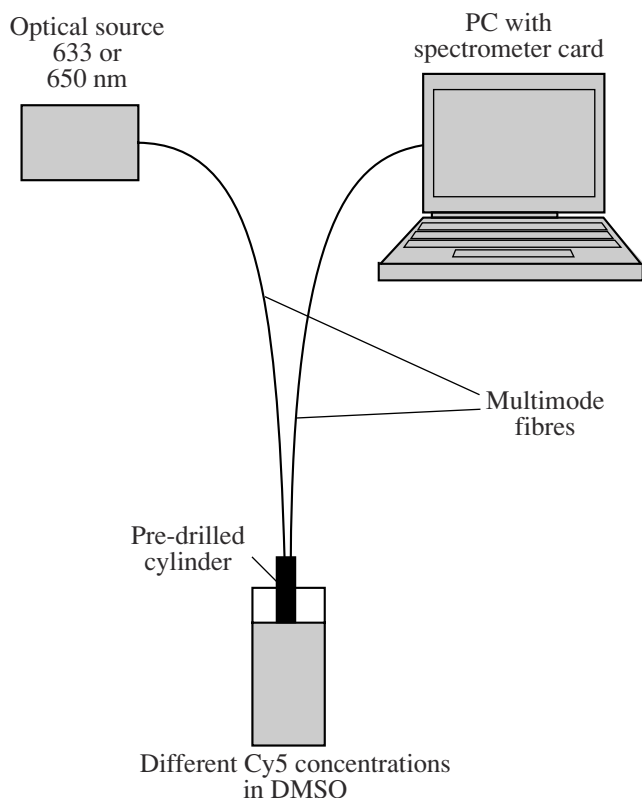


Fig. 1. Experimental set-up (two kinds of optical sources are used).

λ : 650 nm) adapted to the fluorescence particles (Cy5) illuminates the input side of the fiber. The light is guided to the output side and impinges onto the fluorescent medium. The fluorescence light is then collected by the other fiber and detected by a microspectrophotometer (Ocean Optics, 2000 model). Suited digital signal processing is made with Matlab® software.

The functionalization of the vesicles was described in a previous paper [12]. In Fig. 2, we present the variation of the detected signal with the dilution of fluorescent molecules. It is obvious that the fluorescence collection is optimized and proves the efficiency of the optical probe. The obtained curve is conventional and illustrates the comportment of the optical probe vs. fluorescence particle concentrations.

3. PREPARATION OF THE BIOCHIP

Three steps structure this part. First of all, it is necessary to bond target DNA to the sample surface with thiol bondings. The aim is to carry out the link of two kinds of target DNA strands named A1 (AGT-TCTTTGATCAGGTCAGTGTGACCGAACA) and A4 (AGTTCTTTGATACGTCCCATCAAGTCAC-GAC). Their respective probe DNA strands are A2 (AAACTAGTCCAGTGACACTGGACTTGAACGA) and A5 (AAACTATGCAGGGTAGTTCAGTGCT-

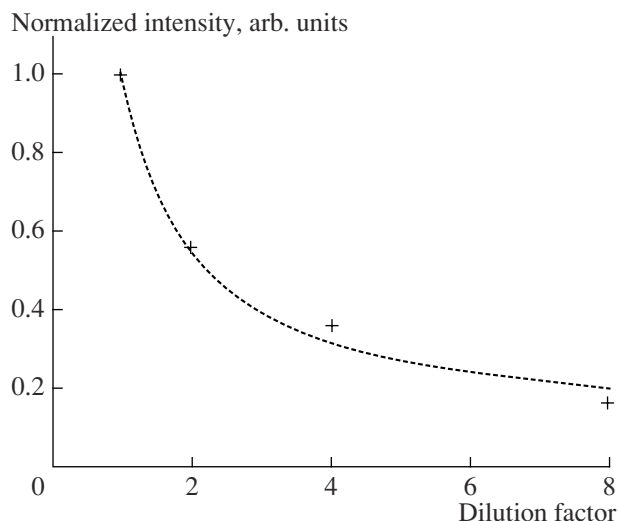


Fig. 2. Fluorescence intensity vs. molecule dilution (normalized ordinates vs. dilution factor).

GAACGA). Note the latter are functionalized with a fluorescent molecule, Cy5. The Fig. 3 illustrates the process of thiolated DNA self assembling. The two targets DNA are linked to two gold plates and the probe DNA are placed to contact with the probe ones by using a liquid flow as it will be described further.

A gold layer is deposited onto the glass plate. To elaborate the biochip, we used 288 nM of A1 DNA in 1 ml phosphate solution and 140 nM for A4, to have homogeneous solutions vs. mass. To bond the DNA, the operating mode consists in diluting Oligo-SH to 10 μ M (be 35 μ l of A1 in 965 μ l of phosphate solution and 71 μ l of A4 in 929 μ l of P). The gold coated glass plates are then washed with solvents (EtOH/H₂O/OG/H₂O). This step is the critical part. To bond the DNA, the glass plates are incubated with the Oligo-SH solution either with A1 or A4 during 120 min in Petri boxes. After this, the plates are washed using P/OG/P/H₂O.

To certify that target DNA is correctly bonded to the gold surface, the experiment was checked by a commercial Biacore™ SPR device. It allows a real time monitoring of the reaction. The result is presented in Fig. 4. It is the plasmon signal from the beginning of the A1 (or A4) injection onto the plate until the wash up of the plate. The signal increases with time for both cases. At time $t = 3600$ s, the injection is stopped and OG solvent is introduced to wash the plate. After this, the plasmon signal at the end of the experiment is 970 RU and 930 RU higher than at its ignition for A1, and 820 RU for A4. RU is the unity used by Biacore™. It is related to the mass of molecules bonded to the surface. These results indicate that target DNA is effectively bonded to the gold surface. If not, the plasmon signal would have been dropping down to its initial value, be 0 after washing.

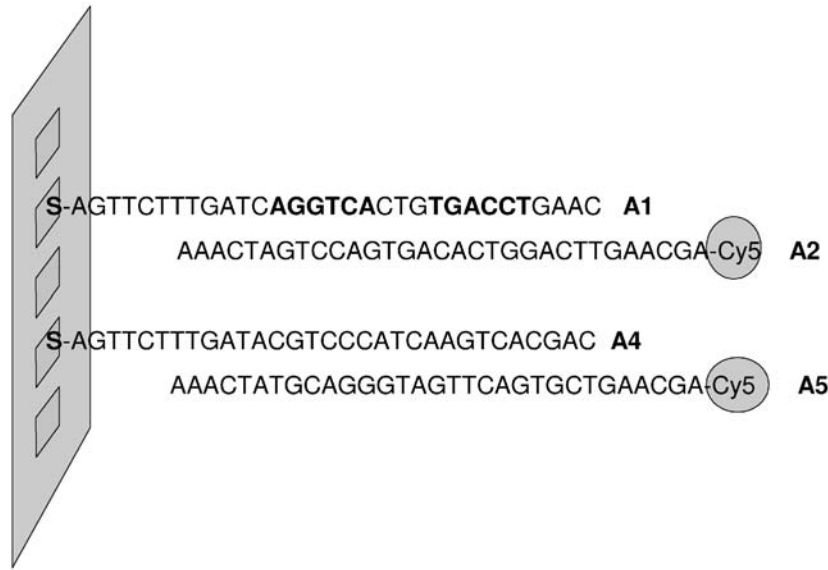


Fig. 3. DNA hybridization process (probe DNA are A2 and A5, target DNA are A1 and A4).

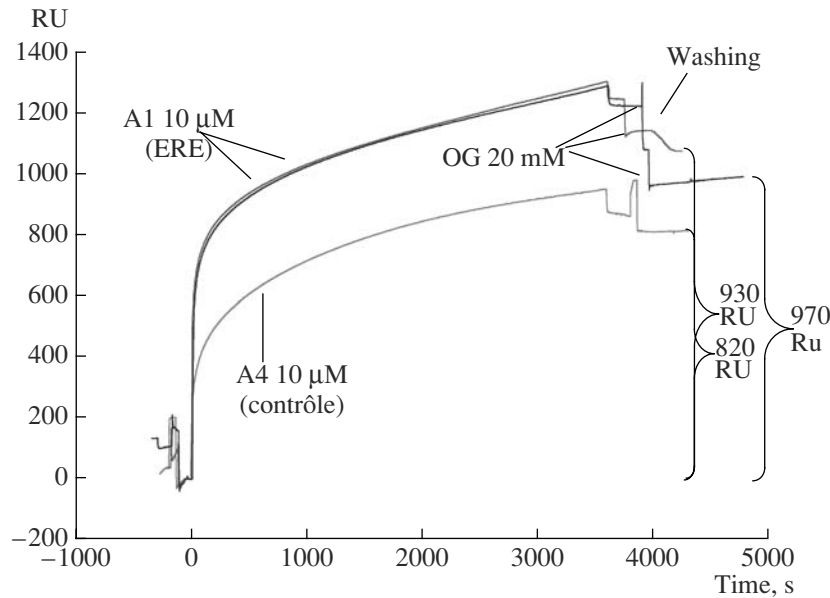


Fig. 4. SPR signal during bonding of target DNA to the gold surface (signal in RU vs. time in seconds).

Next step is to test hybridization of A2 and A5 to A1 and A4 respectively. We achieved this by using a commercial dedicated system located at CLIPP, in the Burgundian part. We used 81 nM of A2 in 810 μ l of phosphate solution and 95 nM of A5 in 950 μ l of solution. The operating mode for hybridizing is: dilutions for obtaining probe oligo solutions at 100 nM (pH 7.4, NaCl 0.25 M), incubation of the functionalized plates with probe oligo solutions during 20 min, wash up with

phosphate (pH 7.4, NaCl 0.25 M). The John device is used at this step.

We tested the hybridization with the confocal microscope dedicated to hybridization detection. We present in Fig. 5 the picture of the plate A1 and the probe A2. A fluorescence signal is clearly visible indicating hybridization. The weak fluorescence intensity seems to come from a possible quenching due to the gold layer.

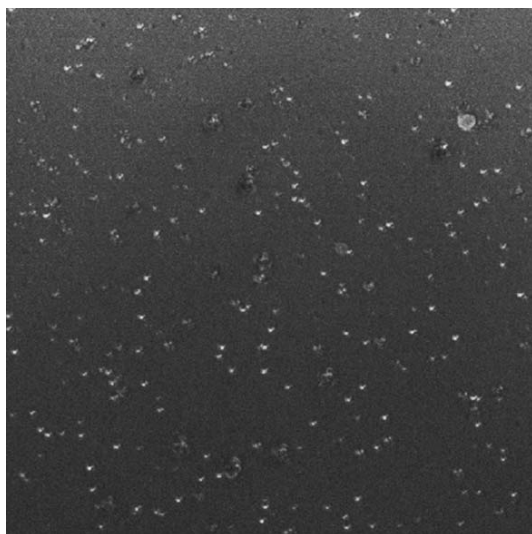


Fig. 5. Confocal microscopy picture of the biochip after hybridization ($0.5 \times 0.5 \mu\text{m}^2$).

We confirmed the hybridization by monitoring the reaction with SPR. Figure 6 gives the plasmon signal vs. time. After washing, the respective remaining signals are 270 RU for A2 and 200 RU for A5. Taking into account the plasmon signals for probe DNA hybridizing versus plasmon signals for target DNA bonding, an estimation for the hybridization rate $270/930$, be 29% for the couple A1/A2 and $200/820$, be 24.5% for A4/A5 can be deduced. As far as we know, with this protocol, we did not get any non specific result. After washing, the probe DNA which is not hybridized with target is removed by the flow.

4. FLUIDIC SYSTEM AND EXPERIMENTAL RESULTS

In order to avoid complicated system for hybridizing as the one used previously, we developed our own microtank for processing the biochip. This microtank is intended to maintain the chip in a target DNA solution volume of 500 μl permanently and assuring a constant flow to renew the DNA. We used silicon and stainless steel to make it rigid and solid. After elaboration of the tank itself by Deep Reactive Ion Etching, the silicon tank is inserted in a stainless steel piece for convenience and solidity. A picture of the tank is presented in Fig. 8. This microtank is very easy-to-use and can be widely used by technicians for improving the contact between probe and target DNA.

The optical probe is placed right above the biochip and immersed into the liquid. It thus increases the collecting power of the probe vs. in air (the NA is multiplied by around 1.33). The optical source is in this case a He-Ne laser ($\lambda = 632 \text{ nm}$, $P = 2 \text{ mW}$). Concerning spatial considerations, the spot size in diameter can be evaluated by calculation to about $800 \mu\text{m}$, be roughly a power of $0.5 \text{ mW}/\text{mm}^2$.

We used two plates (one with target DNA A1 and the other A4). We tested the setup for each plate with the two probe DNA. In Fig. 9, we plotted the four recorded spectra (top left: A1/A5, top right: A1/A2, bottom left: A4/A2, bottom right: A4/A5).

The A1/A2 and A4/A5 spectra clearly exhibit a strong fluorescence signal (660–720 nm) indicating hybridization. On the other hand, the two other results do not show fluorescence but even an absorption in the fluorescence range. We explain this by an extra absorption of either biochemical substances or buffer. How-

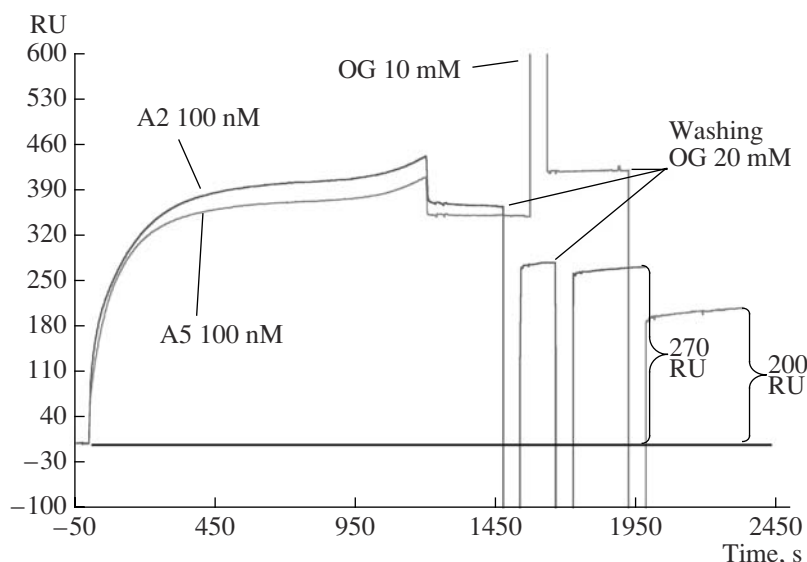


Fig. 6. SPR signal of A1 and A4 plates during hybridization (signal in RU vs. time in seconds).

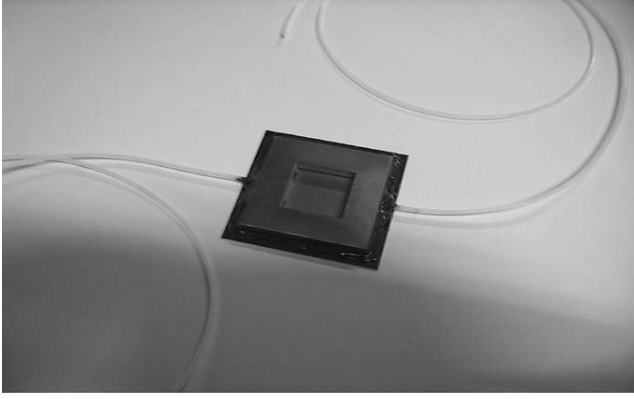


Fig. 7. Microtank picture equipped with capillaries.

ever, the hybridization is clearly underlined. The signal-to-noise ratio can be estimated to more than 10, what is relatively comfortable for interpreting the fluorescence signal.

Compared to the confocal microscopy picture, we can assess that our system is as sensitive as microscopy but it suffers from a main drawback, be the point measurement instead of a 2-dimensional one. However, in most cases, a point measurement is sufficient for hybridization detection and it gives thus all its interest

to the developed optical probe. The fluorescence signal seems strong enough to replace in the future the spectrometer with a conventional photodiode associated to a suited interference filter.

5. CONCLUSIONS

The detection of DNA hybridization is of growing interest in several cases: gene detection, criminal investigations, etc. Generally, it deals with QCM or any way of detecting a bonding of two DNA strands. A widely used method is fluorescence and biochips. We presented here a simple and low-cost way for measuring fluorescence with an integrated optical probe. The elaboration of the biochip has been presented as well as a microtank in which hybridization occurs. The experimental results with a strong fluorescence signal give all its interest to the setup. Our system, coupled to the developed microtank, avoids any complicated step for detecting hybridization. However, it suffers of its spatial characteristics, be the point measurement. Therefore, the next step is to parallelize the detection by using microsystems and notably a microlens array we are currently developing. Associated to it, an interference filter for eliminating the source spectrum and a CCD sensor will perform a parallelization of the hybridization detection.

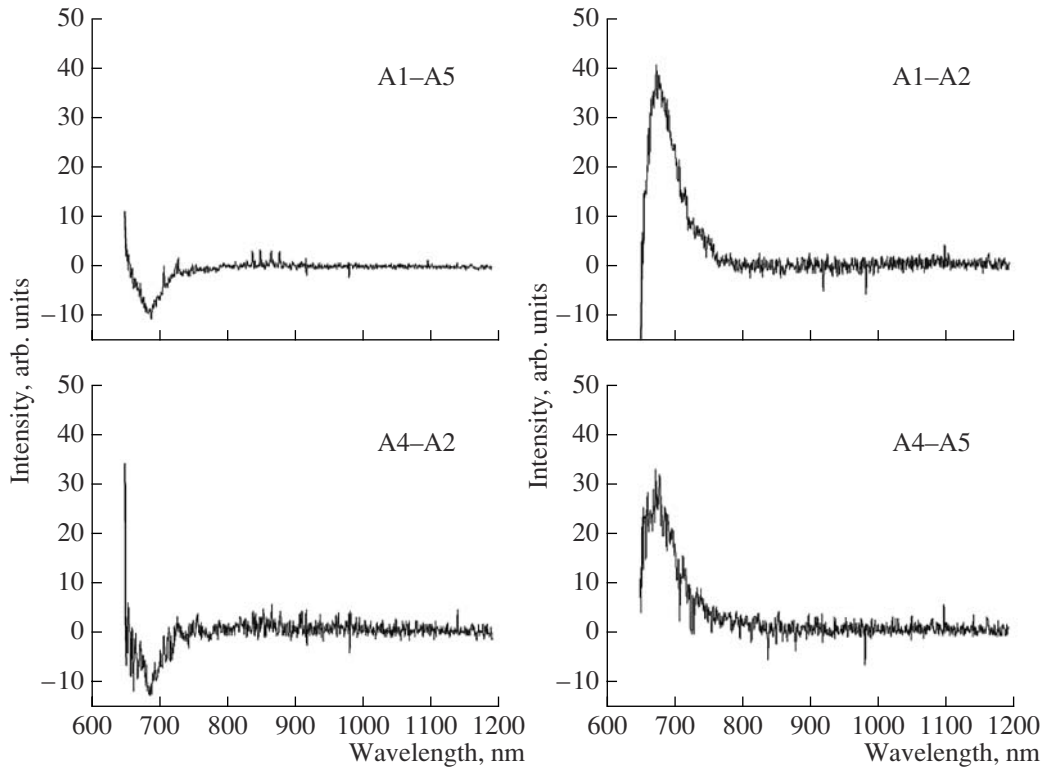


Fig. 8. Fluorescence signals of no hybridized DNA (left) and hybridized DNA (right). The intensity is in arbitrary units, abscissa is wavelength in nanometers.

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