"BioChips" : DNA Microarray Technology - fabrication and applications

Biosensors and High-Throughput Analysis

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Abstract

Micro-arrays are a technology where nucleic acids are bound to a surface and are used to measure the relative concentration of nucleic acid sequences in a mixture via hybridization and subsequent detection of the hybridization events. [1] The latter has become important in gene expression analysis, transcription factor binding analysis, genotyping among others. The objectives of this practical are to use this technique in order to characterize the hybridization of a DNA probe with its fluorescent labelled complementary target. DNA micro-arrays will first be grafted on an aminosilane-coated slides. After which, the DNA micro-array will be hybridized with two different fluorophore-labelled target. Finally, the identification of each DNA molecule will be performed by the fluorescence pattern obtained with a fluorescence scanner. Then, the scanning measurements in order to conclude on the ability and efficiency of DNA micro-array to detect a DNA sequence of interest will be discussed.

Keywords: DNA, Micro-arrays, Fluorescence, Biosensors, Hybridization, Chemical functionalization, DNA Probes

1 Introduction

A DNA microarray, that can be also called a biochip is an assembly of DNA spots on a surface. Each spots contain a specific DNA, in the practical they are called the probes. Other DNA sequences are on disposal that will hybridize to these probes; the targets. To detect whether the hybridization happened or not, fluorescence with fluorophores detection is used [2]. The basic principles of it can be sum-up in Fig 1

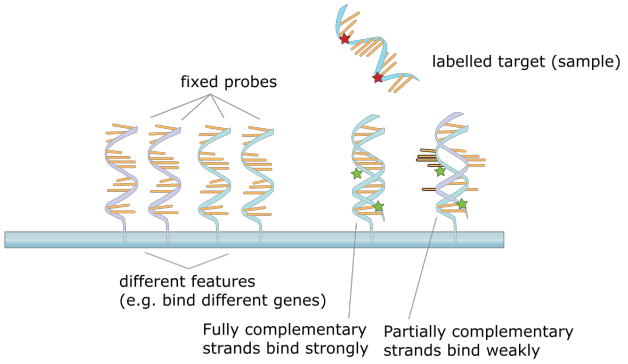


Figure 1: Scheme of how the targets bind to the DNA probes if complementary sequence. Adapted from [2]

DNA microarray have many applications. One of them is the gene expression analysis [1]. It consists of amplifying the messenger RNA (mRNA) which contain the information to do the RNA translation into protein. DNA microarray can be used to identify genes that have different expressions in response to pathogens or an infection [2].

Then the scientific question that have to be answered is : **Can DNA microarrays be a solution to detect specific sequence of DNA with accuracy and specificity ?**

The first part of the practical will be to print DNA probes in a microarray on the glass slide. Then there will be the hybridization of the targets and then the fluorescence analysis to quantify whether the hybridization went well and how

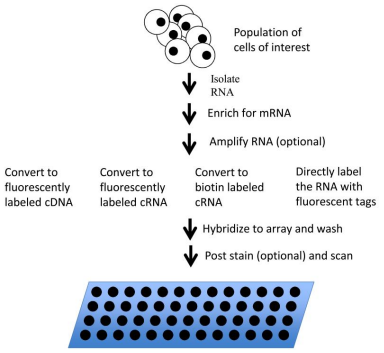


Figure 2: Gene expression analysis via microarrays. Adapted from [1].

specific is the binding. At the end, after our analysis, robust results were obtained to affirm that DNA microarrays can be used to detect DNA sequence of interest.

2 Materials and Methods

2.1 Materials

The probes and the targets have the following oligonucleotides sequence, Fig 3.

The sequence of the oligonucleotide DNA probes are:

Std ("standard" probe)	NH_2-TTTTTGATAAACCCACTCTA
1M (Std-P with one mismatch)	NH_2-TTTTTGATAAAGCCACTCTA
2M (Std-P with two mismatches)	NH_2-TTTTTGATAAAGACACTCTA
X ("unrelated" probe)	NH_2-TTTTTTTTTCCAAGAAAGGACCCG

The sequence of the fluorescently labelled DNA targets are:

Std-T ("standard" target)	Cy3-CATAGAGTGGGTTTATCCA (pink)
2M-T ("2M" target)	Cy5-CATAGAGTGICTTTATCCA (blue)

Figure 3: Sequence of the oligonucleotide DNA probes and the fluorescently labelled DNA targets. Adapted from [3].

The fluorophores used are Cy3 and Cy5 and they have these distincts excitation and emission spectra, Fig 4. They will be binded to the targets according to Fig 5.

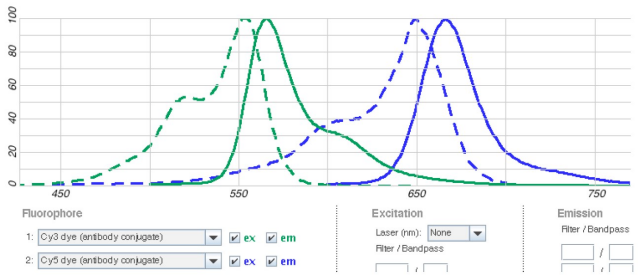
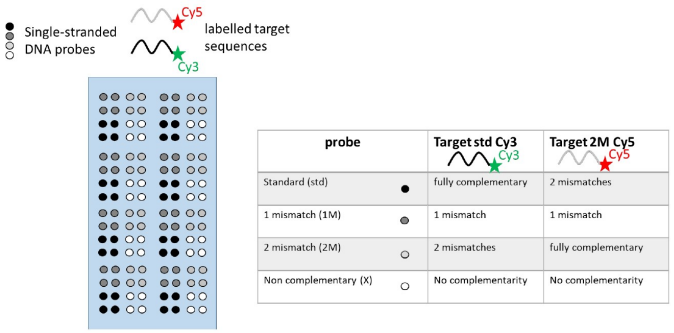


Figure 4: Excitation and Emission spectra of Cy3 and Cy5 fluorophores.



probe		Target std Cy3	Target 2M Cy5
Standard (std)	●	fully complementary	2 mismatches
1 mismatch (1M)	●	1 mismatch	1 mismatch
2 mismatch (2M)	○	2 mismatches	fully complementary
Non complementary (X)	○	No complementarity	No complementarity

Figure 5: This is how the fluorophores probes of Cy3 and Cy5 have been disposed on the microarray. Adapted from [3].

2.2 Chemical functionalization on the glass slide

2.2.1 Re-activation with 0.1 M KOH

Re-activation was done with 0.1 M KOH. 50 mL of KOH 0.1 M was prepared by adding 3 pellets (appoximatly 280 mg) of KOH in a gratitude cylinder with 50 mL of ionized H2O. The solution was sealed with a parafilm and homogenize. Amino-silanized glass slides was incubated in a plastic box during 5 minutes in the 0.1 M KOH solution. After the incubation the slides were rinsed in ionized H2O.

2.2.2 Coupling with glutaraldehyde

The coupling with glutaraldehyde was performed working under a fume hood wearing gloves. 10% glutaraldehyd was prepared in a gratitude cylinder. Firstly, 30 mL of ionized H2O was added to the gratitude cylinder and secondly, 20 mL of 25% glutaraldyhyd was added to the cyliner. The solution was homogenized. The slides was transferred to another box on a shaker and the slides were incubated in the 10% glutaraldehyd solution during 10 minutes. Further, the slides were rinsed twice with water in the fume and twice afterwards in the zinc. After this the slides were transferred by to the other box. The slides were put on a piece of paper and dried with an air gun.

2.3 Arraying of DNA probes

2.3.1 Preparation of the DNA probes

The 100 µM dilution of probes to be spotted was prepared at the bench. The probes were distributed into a 384-well micro plate with the following distribution: Std-P, 1M-P, 2M-P, X-P in wells A1, A2, B1, B2 respectively [4].

2.3.2 DNA probes spotting

The glass slides and the 384-well micro plate containing the probes were placed in the robot. The spotting procedure was ran. A humid chamber was prepared on the bench by taping soaked paper inside the plastic box. The slides were incubated in the humid chamber during 10 minutes at room temperature. The leftover DNA probes from the spotting procedure was recovered and the 384-well micro plate was washed. At the end we obtain this configuration 5.

2.3.3 Chemical stabilization

50 mL of NaBH4 0.35 % was prepared under a fume hood by firstly adding 50 mL of water to a gratitude cylinder and then 0.175 g of NaBH4 was weight on an analytic balance weight. The solution sealed with parafilm and homogenized. The 0.35 NaBH4 solution was poured onto the slides in the box and the slides were incubated during 5 minutes at room temperature. After the incubation the slides were rinsed twice in water. The slides were dried and put into 50 mL tubes. The tubes containing the slides were centrifuged during 4 minutes at 200 x g. After this procedure the tubes containing the slides were put in the fridge for one week.

2.4 Hybridization of samples to the DNA microarray

2.4.1 Preparation of the sample and of the target mix

For the experiment prelabled fluorescent DNA targets were utilized. The DNA targets utilized were Cy3-Std-T (pink) and Cy5-2M-T (blue) each bought in a stock solution with a concentration of 100 µM. Each of the stock solutions were diluted by adding 2 µM of target to 0.3 M NaH2PO4. This was done prior to our experiment.

The preparation of the target mixtures were performed by adding 10 µL of each target to one 0.5 micro-tube each. The targets were diluted to 0.2 µM by addition of 100 µL of 1x Hybridization buffer to each 0.5 micro-tube respectively.

2.4.2 Hybridization of the sample

The DNA microarray, the 2x Hybridization solution and the sample solution were preheated in the oven at 40 degrees during 10 minutes. A humid chamber was prepared from a 50 ml tube by adding 2 ml of water to it. The 100 micro L of the sample was dispensed on the microarray and it was spread by adding a glass cover slip to the solution. The microarray was put into the humid chamber without touching the water and it was incubated during 10 minutes in 40 degrees.

A strigent wash was executed on the DNA microarray by washed it in 20 mL of 2x SSC at room temperature during 2 minutes. During this time it was shaken gently by hand and the cover slip was removed with tweezers. Further, the DNA microarray was dried by centrifuging it in a 50 mL tube during 4 minutes at 200 x g.

2.5 Measurement and Data analysis

DNA Micro Array was scanned with the AmpliReader machine that is jointed with the Array4D software. Once, the scan is done a grid is applied on it, where every square will contain one spot, it will be useful for our analysis.It can already underline major information. **Mean, Median, Standard Derivation, Coefficient of Variation, Signal-to-Noise Ratio (SNR)**.These data are available for each fluorescent channel, the 532 nm channel (Cy3) and the 635 nm channel (Cy5). These data will be used to observe the hybridization effect of the DNA microarray.
To plot the mean intensity of fluorescence regarding each square was decided as depicted in Fig 6 to do our analysis.

1 Cy3 1MM Cy5 1MM	2 Cy3 1MM Cy5 1MM	3 Cy3 2MM Cy5 FC	4 Cy3 2MM Cy5 FC
5 Cy3 1MM Cy5 1MM	6 Cy3 1MM Cy5 1MM	7 Cy3 2MM Cy5 FC	8 Cy3 2MM Cy5 FC
9 Cy3 FC Cy5 2MM	10 Cy3 FC Cy5 2MM	11 Cy3 NC Cy5 NC	12 Cy3 NC Cy5 NC
13 Cy3 FC Cy5 2MM	14 Cy3 FC Cy5 2MM	15 Cy3 NC Cy5 NC	16 Cy3 NC Cy5 NC

Figure 6: How squares of a block are labeled for our analysis.

3 Results

What was obtained with the scanning robot is in Fig 7. Only, the two first rows of the grid were kept for the analysis as our microarray [5] is the one that have been double spotted by the robot MicroGrid II after it crashed.

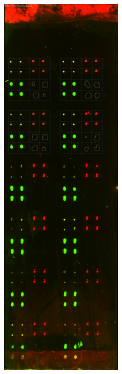


Figure 7: The Microarray after the Scanning. It is clearly visible which spots have been targeted by the fluorophores and which one not. To validate this visual look, detailed analysis has to be investigated.

3.1 Fluorescence intensity analysis

3.1.1 F635 intensity analysis

The first major information that will be emphasized is **specificity**. If Cy5 analysis is considered (F635 nm channel), It can be found that the mean signal intensity is consistently stronger when there is perfectly complementary between the probe and the target. It can be observed in Fig 8 with the intensity of the F635 Means over the arrays. Here the measured fluorescence intensity for squares 3, 4, 7 and 8 is more than 12000 higher than the others. It is even more obvious on Fig 9 where the decrease of intensity regarding as the number of mismatches increases is observed. These measurements are **sensitive**.

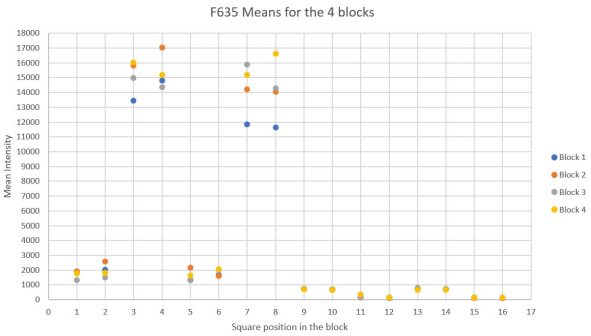


Figure 8: Plot of the fluorescence mean intensity at 635 nm in the four blocks.

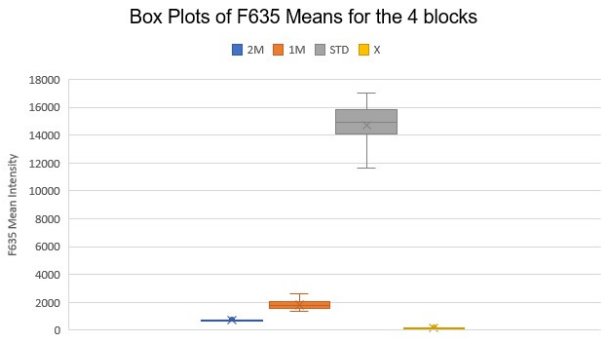


Figure 9: Boxplots of the four blocks with the F635 intensity.

3.1.2 F532 intensity analysis

Fig 10 represents the intensity of the F532 channel over the arrays (Cy3). It is observed that the fluorescent intensity is stronger when the probe is perfectly complementary (Intensity-8000) with the target than when there is no binding (Intensity-200). It is approximately 94 times more intense for F532 intensity between STD and X probes. Furthermore, the signal consistently decreases when there is increased mismatches between the probes and the targets. It is underlined that when the probe has only one mismatch (1/2/5/6) Fig 10 the signal is low (around 3000) but still higher than when the probes have more mismatches (no more than 500), it means that it has partially bound. It is also further described in Fig 11. There is a nice **reproducibility** between the four blocks as the intensity is always around the same value (mismatches related) and a nice **repeatability** between the spots of a same block.

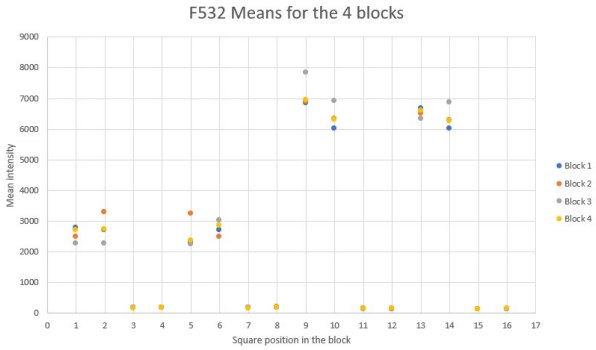


Figure 10: Plot of the fluorescence mean intensity at 532 nm in the four blocks.

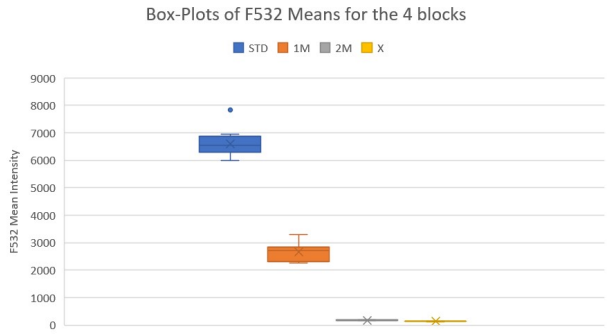


Figure 11: Boxplots of the four blocks with the F532 intensity.

3.2 Consistency of the results

The tab in 12 sum-up our previous analysis.

	STD	1M	2M	X	N (number of spots)
Average of F532 Mean	6599,6±457,9	2653,2±334,7	175,4±8,7	136,2±7,9	16
Average of F635 Mean	715,2±35,4	1798,6±345,4	14708,3±150	154,9±54,6	16

Figure 12: Tab doing the sum-up of the measurements with the standard deviation as the discrepancies.

To check whether these results are consistent, the percentage of pixels in the spot with a fluorescence level above the "the background mean" + 1 background standard deviation were plotted to see whether the analyzed data and the spot has any meaning, Fig 13. It is observed that the spots number 11/12 and 15/16 has a percentage close to 0, indeed these are the DNA spots with no complementarity at all, so there will not even be partially binding. Hence, it shows that the detection level is accurate.

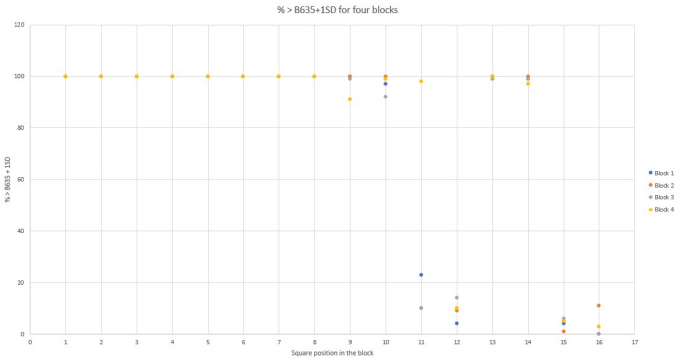


Figure 13: Plot of the percentage of pixels in the spot with a fluorescence level above the background mean

4 Discussion

To check if we obtain the same results with more blocks, the data of the group of Diego and Anass that had more blocks than us were analyzed and it has been observed the same trends in the hybridization Fig 14; high intensity (17000) for the complement probes, less for one mismatch (2500) and lower for two mismatches (500). It can be argued that this protocol is really robust as **reproducible** results between the blocks and between the groups were found.

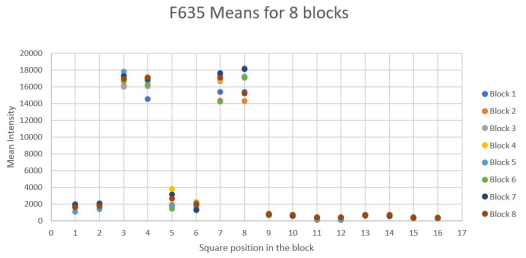


Figure 14: Plot of the fluorescence mean intensity at 635 nm with more blocks at our disposal

The mean fluorescent intensity was analyzed. To go further, to remove the background intensity to have more "accurate" data is smart. Even if the trend is the same, Fig 15. The coefficient of variation is also a good mean to check whether our results are consistent as it indicates the size of a standard addition in relation to its mean, so the higher, the greater the dispersion level around the mean will be [6], Fig 16.

In addition, the **signal to noise ratio** in Fig 17 was analyzed , and it validates our previous results that when there is a complementarity the SNR is high (between 40 and 160), so the signal is more important than the noise. When there are mismatches, this value decreases meaning that noise from the background is more prevalent.

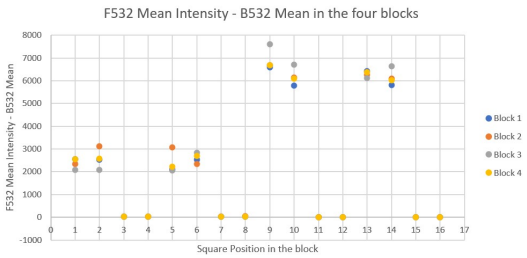


Figure 15: Mean Intensity minus the background mean at 532 nm.

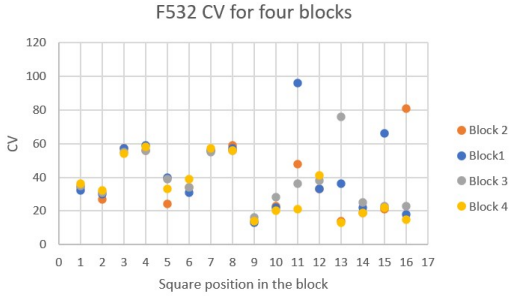


Figure 16: CV of the channel F532 nm for the four blocks. When there are mismatches, CV are higher than the complementarity probes, showing that fluorescent is less focused. It validates our results.

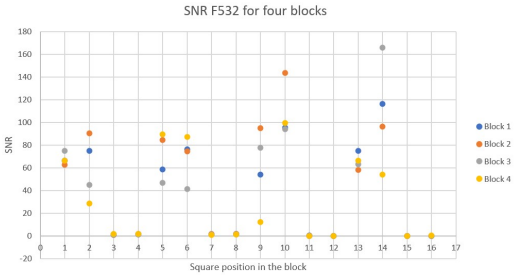


Figure 17: Plot of the signal to noise ratio at 532 nm.

As in Fig 17 channel F532 was studied, SNR is high for squares 1/2/5/6 and squares 9/10/13/13, respectively one mismatch and standard target, it is consistent. However, the repeatability between spots is not that good as SNR can be in a range from [55-165] for square 14 for example.

5 Conclusion

With this practical, the fact that DNA micro-arrays is a good method to determine the complementarity between two DNA strands was showed. Indeed, when the targets and the probes are hybridizing, the fluorescent intensity measured is clearly more relevant than when it does not bind or only partially. It answers our initial problematic : **DNA microarrays is a good solution to detect specific sequence of DNA with accuracy and specificity.** With this practical, the interest of this method and why it can be used for other genomics applications were understood.

References

[1] R., Bumgarner: *Overview of dna microaarays: types, applications, and their future*. 2013.

[2] Wikipedia: *Dna microarray*. 2022. https://en.wikipedia.org/wiki/DNA_microarray.

[3] Marianne Weidenhaupt, Franz Bruckert, ALexandre Dawid: *"biochips":dna microarray technology: fabrivation and applications*. 2022. https://www.esonn.fr/.

[4] geneWave: *amplislide - high sensitivity amino or epoxy coated double color microarray slides*. 2008.

[5] geneWave: *amplireader - compact ccd-based microarray reader*. 2007.

[6] Hayes, Adam: *Co-efficient of variation meaning and how to use it*. Investopedia, 2022. https://www.investopedia.com/terms/c/coefficientofvariation.asp.