Simulation, fabrication and characterization of microlens oriented fluorescence detection systems for DNA microarrays

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We explored the use of microlenses in commercial and miniaturized low-cost detection systems for the analysis of fluorescence in DNA microarrays. We investigated through simulations the properties of trans- and epi-illumination detection configurations and analyzed how microlenses can increase the detection efficiency. We fabricated microlenses by means of our in-house rapid prototyping technology called deep lithography with protons and we used them in a demonstration setup to compare our simulations with experimental data. During this conference we will overview novel designs for miniaturized, parallel fluorescence detection systems, which can be fully implemented while using the strengths of deep proton lithography.

Introduction

Biology, and more specific molecular genetics, will probably become the science of this century. The knowledge of genetic properties of organisms, which are coded in DNA sequences, will be of great importance for the development of new therapies. Microarrays are undoubtedly the bioanalytic instruments of the future to investigate our DNA profile in one single step [1]. They consist of an array of spotted DNA probes fixed on a microscope slide. Each probe with its specific DNA sequence can make a double strand with only one characteristic complementary DNA sequence, which may be present in the investigated sample on top of the slide. This selective formation of double strands is called hybridization. As the complementary DNA strands in the sample are marked with a fluorescent group, information is available about the genetic properties of the organisms after hybridization. The power of microarrays lies in the ability to investigate a complete DNA profile in a parallel way. Despite its advantages microarrays can offer the medical science, a full integration towards the family doctor's office is still not foreseen for the next year and so this technology remains only available to specialized laboratories. One of the reasons is the low efficiency of the hybridization analysis: due to the diffuse nature of the fluorescent light of the marked DNA strands, high detection efficiencies can only be obtained by use of expensive, time-consuming detection methods. In this paper, we will investigate the use of microlenses to direct more light towards the detector (Figure 1).

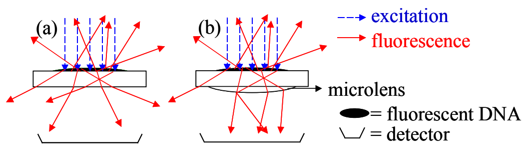

Figure 1: Detection of fluorescent signal (a) without microlens, (b) with an integrated microlens.

First, we will discuss the integration of microlenses in present commercial microarray scanners to increase their efficiency. Moreover, in this work we will make a first step towards mass fabricated low cost detection systems for rapid parallel DNA analysis with integrated plastic microlens arrays.

Fluorescence detection in commercial scanners

Most of the present commercial microarray scanners operate according to a confocal epi-illumination detection setup. A strong microscope objective creates a focused beam for the excitation of small pixels, and by scanning the slide the total image is constructed. During this work, we had the opportunity to scan slides with an Agilent G2565AA Microarray Scanner at the MicroArray Facility in Leuven [2]. This scanner provides two resolutions: 5 or 10 microns per pixel. Furthermore, this scanner also contains an autofocus system, which adjusts quasi continuously the position of the slide during the scan, in order to focus the excitation beam as strong as possible on the spotted surface of the slide. We investigate the integration of microlenses in the epi-illumination setup in Figure 2. The incoming excitation power (P_{in}) is focused on the fluorescent spot. Without a microlens, a certain cone of fluorescent light (gray) can be collected by the objective (P_{out}), according to its numerical aperture (NA). As shown in Figure 2a, by means of the microlens, more fluorescent light (black) can be collected and the efficiency of the system increases, providing that the autofocus system can adjust the position of the slide. However, the situation changes if we work with microlenses with a small diameter and/or objectives with a large NA. This is a more realistic situation, as commercial scanners use objectives with a large NA in order to collect a large angle of fluorescent light, and small microlenses are desired to integrate as many as possible on one slide. Present DNA microarrays formats use spot sizes of 110µm with a pitch of 175µm [2], so microlens arrays with similar properties are desired. As shown in Figure 2b, not all of the incoming light originating from the objective is focused on the spot. Moreover, the acceptance angle of collected fluorescent light is not any longer significantly greater than without microlens. The efficiency of the system will therefore decrease.

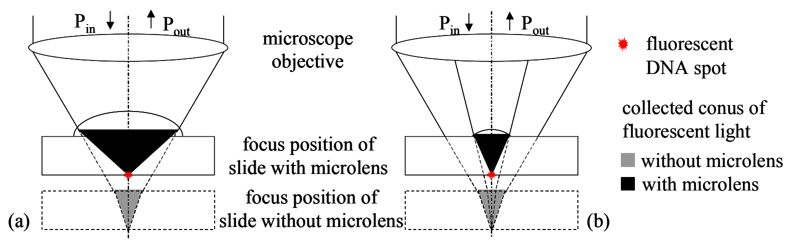

Figure 2: Detection in epi-illumination scheme with microlenses: (a) microlens with large diameter, (b) microlens with small diameter.

We illustrate what happens in this second case with the incoming excitation power by means of sequential ray-tracing simulations in Figure 3a/b. We divide the incoming excitation beam in 2 parts: (a) the rays which are collected by the microlens and focused on the spot (①), and (b) the excitation rays which pass next to the microlens and which are not focused on the backside of the slide (②) but only behind the slide (③). Also the results in Figure 3c obtained with a setup built in the lab show that only the rays collected by the microlens will focus on the backside of the slide (①, shaded part). The other rays pass next to the lens and are only focused 2mm behind the slide (②,③).The microlenses used in this setup were fabricated by means of Deep Lithography with Protons, a technology for rapid prototyping of micro-optical components, optimized at the Department of Applied Physics and Photonics of the Vrije Universiteit Brussel [3].

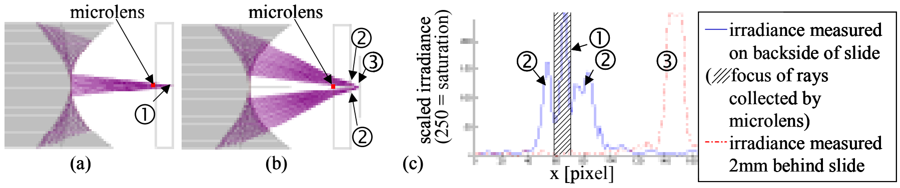

Figure 3: (a) Ray-trace of excitation rays collected by microlens and focused on DNA spot, (b) ray-trace of excitation rays passing next to microlens and focused at 2mm behind slide, (c) results from lab setup: irradiance measurements on backside of slide and at 2mm behind slide.

Yet there are two ways of using microlenses with diameter smaller than 150µm in similar configurations without decreasing efficiency. By integrating microlenses on thinner microscope slides, Figure 4a/b, we will be able to focus all the excitation light onto the spot. By choosing a suitable focal length, we can then increase the acceptance cone of collected fluorescent light. Figure 4c shows the simulation results performed with a sequential ray-tracing program (SOLSTIS) where we investigated the impact of the lens plate thickness on the efficiency of the system using an objective with a NA of 0.62. We observe that the use of microlenses with diameters between 100 and 150µm on microscope slides with a thickness of 150µm enables to focus all the excitation light originating from the objective on the DNA spot.

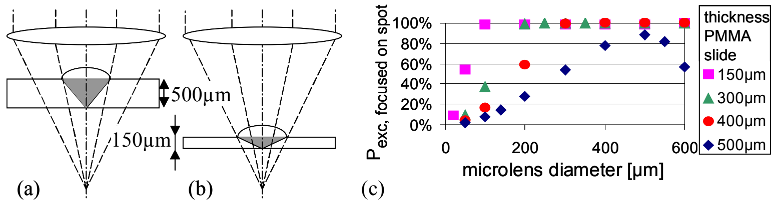

Figure 4: (a,b) With microlenses on a thinner slide, all the incoming light can be collected. (c) Simulation of the collected incoming excitation light in the DNA spot as a function of the microlens diameter, for several slide thicknesses.

On the other hand, as illustrated in Figure 5a/b, we can create a cheaper detection system with comparable efficiency to present commercial systems, by combining a cheaper objective with smaller NA and a microlens array. Again this is illustrated by means of simulations in Figure 5c. The slide thickness remains fixed on 500µm. Although an objective with smaller NA can collect only a relatively small cone of fluorescent light, in combination with a microlens it can achieve significantly larger efficiencies. (e.g. the combination of an objective with a NA of 0.4 and a microlens with a diameter of 500µm, gives an efficiency of 17%, instead of 7% without microlens)

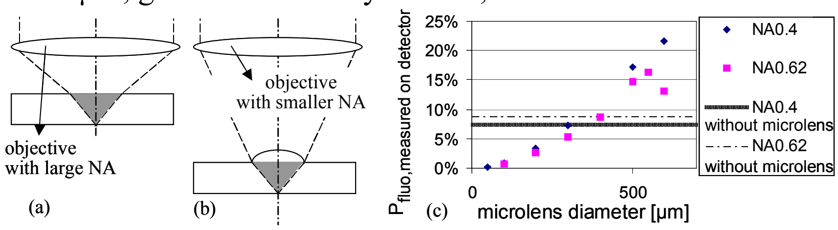

Figure 5: Comparable cones of detected fluorescence obtained with (a) an objective with high NA and (b) an objective with lower NA combined with a microlens. (c) Simulation of detected fluorescence as a function of the microlens diameter, for several NA values of the objective.

Conclusion and future perspectives: miniaturized detection systems

Medical diagnostics still rely upon labor intensive, slow, and expensive laboratory techniques and there still exists a need for small portable miniaturized detection systems that provide immediate point-of-care tests close to the patient, which can be performed by non-laboratory staff. Therefore, rather than integrating microlenses in present commercial scanners, we want to explore novel designs for low-cost, miniaturized, integrated, robust, parallel fluorescence detection systems, which can be fully implemented while using the strengths of Deep Lithography with Protons.

One of the goals of a fluorescence detection system is an effective separation of non-absorbed excitation light and the fluorescence signal. Unfortunately, it is difficult to integrate beamsplitters and dichroic mirrors into miniaturized devices. As a solution, Roulet et al. designed a system that works in trans-illumination instead of epi-illumination and where the incoming excitation beam has an angle of 45° with respect to the slide [4]. Figure 6 shows the proposed off-axis illumination setup, which we will optimize in the future for fluorescence detection on microarrays. The main challenges in such miniaturized detection systems are the alignment and the quality of the optics. Therefore, Deep Lithography with Protons is a promising fabrication technology, as it gives the possibility to monolithically integrate microlenses and mechanical alignment elements by which a perfectly aligned stacked detection system with several microlens plates can be fabricated.

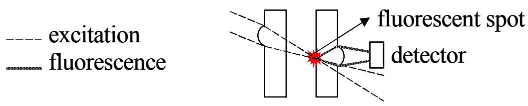

Figure 6: Off-axis illumination scheme.

Acknowledgements

The authors would like to thank Pedro Vynck for the irradiation of the micro-optical components. This research was supported by the Fund for Scientific Research-Flanders (FWO-Vlaanderen), the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-GBOU), the Federal Office for Scientific, Technical and Cultural Affairs in the framework of the InterUniversity Attraction Pole Program "Photonic Information Systems" (DWTC-IAP), the Concerted Research Action "Photonics in Computing", the Research Council (OZR) of the Vrije Universiteit Brussel and the European Commission NoE 'NEMO'. Sara Van Overmeire is indebted to the FWO for her research fellowship and financial support.

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