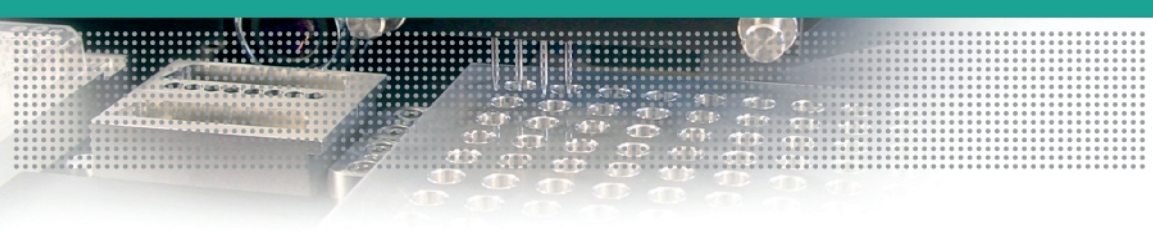

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sciFLEXARRAYER Application Note
No. 08014

DNA and RNA applications on sciPOLY3D microarrays - Multiplex detection of RNA

One major task for the design of microarray based nucleic acid tests is the decision for a platform and substrate material, e.g. polymer microfluidic cartridges, microwell plates, slides or membranes. This choice usually is constrained by the immobilization chemistry. Most conventional immobilization chemistries rely on surface functionalization of the whole substrate with e.g. carboxy or epoxy moieties.
With sciPOLY3D all polymers can be furnished with microarrays without pre-treatment thereof. The only additional process step is a short illumination with UV light after printing. Thus, the whole process of microarray production is reduced to printing and an additional few minutes for irradiation, which can easily be integrated in a high-throughput microarray production line.

Applications with sciPOLY3D include multiplexed assays for detection of microRNA. This class of RNA is non-coding and involved in regulatory processes of gene expression. They are of great significance as diagnostic and prognostic biomarkers, e.g. for clinical cancer therapy. But miRNAs are also a challenge for multiplexed diagnostic assays, since their sequence is often highly similar and the molecules are rather short (~19-24 nucleotides). Together with partners from science and industry Scienion developed a platform for detection of cancer related miRNA patterns (BMBF KMU-innovativ project IMRA, FKZ: 031A094B).
sciPOLY3D permits straightforward implementation of these analyses in a 96 well plate or a fully automated microfluidic cartridge, respectively. In this application a modified isothermal RNA amplification assay (NASBA – Nucleic Acid Sequence-Based Amplification) is combined with microarray based detection in a microfluidic cartridge. In addition to miRNA, other RNA classes such as protein coding mRNA can be detected in parallel in the same assay on the same microarray.

Materials and Methods

Preparation of the sciPOLY3D/DNA microarrays: sciPOLY3D was dissolved in sciPOLY3D SOL1, mixed with sciPOLY3D SOL2D1 and various DNA probes. The mixtures were dispensed with a sciFLEXARRAYER in a microarray format on PMMA (polymethyl methacrylate) slides, 96 well plates and in within the structures of a microfluidic cartridge (Fig. 1). After the spotting process sciPOLY3D microarrays were crosslinked with UV light (1.25 J/cm2 @254 nm) and are immediately ready for use.

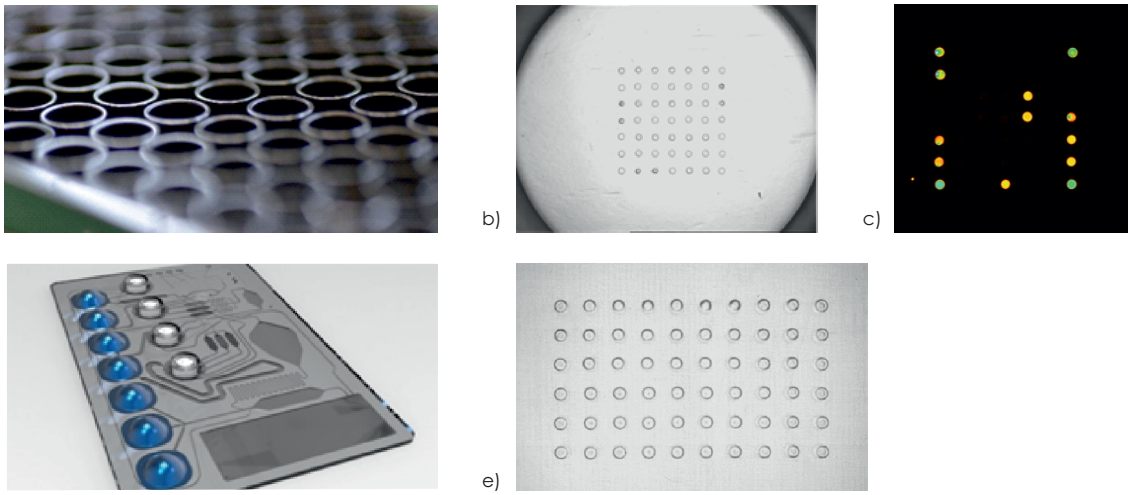

Fig. 1 a) 96 well plate with non-binding properties for low background, b) image of a microarray in a well of a 96 well plate, taken by a sciFLEXARRAYER head cam, c) color-coded fluorescence image of such a microarray after an assay with human miRNA-155; d) schematic depiction of microfluidic cartridge with integrated sample processing including RNA extraction, amplification and microarray-based detection (©microfluidic chipshop), e) image of a microarray in such a microfluidic cartridge.

The RNA samples were prepared from in vitro transcribed and synthesized RNA, respectively and amplified using a universal NASBA assay. The biotinylated RNA products were incubated on the microarrays and stained using Cy5-conjugated streptavidin. Fluorescence detection was realized with a SensoSpot Fluorescence reader (Sensovation AG).

Results

As shown in Fig. 2, the combination of microarray based detection and a multiplex amplification assay, up to 14-plex detection of different RNA sequences was successfully tested. In this case, a combination of breast cancer related mRNA and miRNA parameters were selected. These results show that the approach presented here might offer interesting potentials for applications that require multiparametric and complex analytics of mRNA and small non-protein coding RNA like miRNA in parallel.

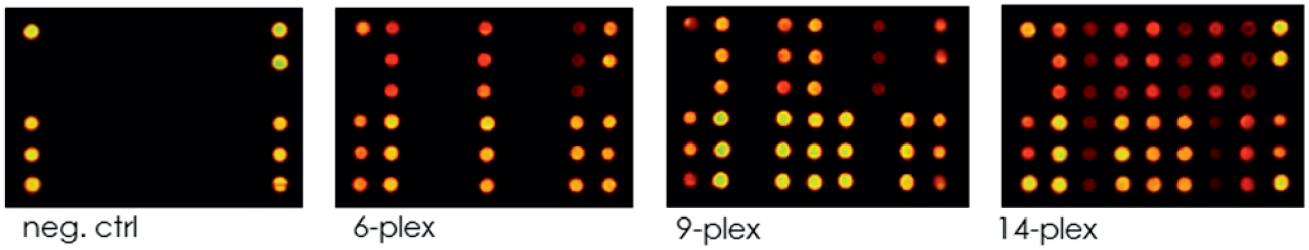

Fig. 2 Examples of multiplex detection of RNA sequences after isothermal amplification and labeling of RNA with a fluorophore. Probe oligonucleotides are immobilized with sciPOLY3D on untreated PMMA.

Summary

sciPOLY3D enables covalent and robust immobilization of unmodified DNA oligonucleotides for multiplex detection of RNA and DNA analytes. The use of sciPOLY3D eliminates all wet chemistry steps in the DNA microarray manufacturing process. This feature facilitates the production of microarrays in structured polymer supports such as standard 96 well plates and customized microfluidic cartridges.

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