Micropatterned Substrates with Improved Uniformity of Deposition for DNA Microarrays

CNF Project # 762-99
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Abstract:

Biomolecular arrays have become a core technology used in numerous fields for parallel analysis. However, it has become obvious that current microarrays are hindered by methodological and technological barriers that limit their sensitivity, reproducibility, and therefore their utility. A fundamental problem with DNA microarrays is the relative insensitivity in detecting weakly expressed transcripts; this is especially significant when examining gene expression in complex tissues. While it has been proposed that a marked improvement in the detection limit of microarrays could be achieved if a uniform DNA layer can be spotted [2], the issue of its implementation has not been fully addressed. In an effort to resolve this problem, we have fabricated micropatterned substrates for the uniform deposition of DNA with a polymer lift-off technique [3]. We pursued a methodology compatible with existing microarray technologies, which constrained the substrate area where the probe DNA were spotted. This yields a uniform layer of deposited DNA and improves the microarray data reproducibility between replicates on a single slide, and also across multiple slides.

Summary:

Micropatterned substrates were fabricated by vapor-deposition of a conformal polymer coating on glass substrates, followed by patterning with photolithography and oxygen plasma reactive ion etching (Figure 1). This process defined the exposed areas of the underlying substrate. The fabricated substrates were spotted with probe oligonucleotides in a conventional microarrayer. The polymer coating was then peeled off from the surface leaving behind only the material spotted within the openings. The substrates were later hybridized and processed in the same way as a conventional microarray glass slide.

To test deposition uniformity, fluorescently-labeled oligonucleotide probes were spotted on microfabricated substrates and control slides, and then imaged with confocal microscopy. It was observed that constraining the available area to the patterned openings facilitated the uniform deposition of DNA, reducing or eliminating the “donuts” seen with commercial glass substrates (Figure 2). To test the impact on sensitivity and reproducibility, we used successive dilutions of an unlabeled gene pool probe, which functioned as a universal positive control binding to RNA from any murine tissue sample [4]. As expected from previous reports [4], no signal was observed on control slides when the DNA was spotted at concentrations below 5 pg/µl. This provides an indication that constraining the deposition surface as well as providing a more hydrophobic region outside the exposed glass areas, produced uniform probe DNA deposition patterns and could dramatically improve the reproducibility of microarray data.

Collectively, these results indicate that arrays spotted on microfabricated substrates are more reproducible than standard arrays. Furthermore, the presence of the polymer during the spotting procedure reduces the background present in inter-spot areas. The combined improvements could allow for faster identification of differentially expressed transcripts in complex tissues. Experiments are under way to evaluate the sensitivity of these microfabricated arrays with real tissue samples.

References:


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Figure 1, left: Fabrication, DNA patterning and hybridization for polymer lift-off microarrays.

Figure 2, below: Fluorescently-labeled oligonucleotides observed with confocal microscopy: a, b) Commercial glass microarrays with most of the signal on the outer rim; c, d) Our microfabricated arrays with uniformly spread signal.

• A major challenge for current microarray technology is the uniform deposition of spotted biomaterial.

• We have improved spotted DNA uniformity by developing microfabricated substrates that constrain deposition to micron-sized openings.

• Comparison with commercial glass microarrays showed that our substrates have comparable sensitivity and improved reproducibility between replicates on a single slide and across slides.