Keysight Technologies
Using AFM to Characterize DNA Microarrays

Application Note

KEYSIGHT TECHNOLOGIES

Introduction

DNA microarrays have proven to be invaluable tools in both clinical and research settings for gene expression analysis, genotyping and to identify mutants in cell populations or tissue samples because they enable highly parallel analysis of thousands of individual DNA sequences [Heller 2002]. Each microarray can contain tens of thousands of unique probes per cm2 which are used to characterize biological samples in a multiplexed manner. In a typical assay, biological targets are isolated, tagged with a fluorescent dye and then introduced to the probes on the microarray surface where hybridization reactions occur between complimentary probes and targets. After the hybridization reactions are complete, microarrays are generally read in a laser-based fluorescent scanner to identify and quantify the number of probe–target duplexes.

3D AFM image of a Keysight DNA microarray

Microarray Probes

Microarrays are composed of discrete probe elements, usually attached to silanized glass substrates Conzone 2004]. Often, the probes are oligonucleotides, which are relatively short synthetic DNA molecules, Keysight Technologies, Inc. Microarrays but microarrays can contain longer, enzymatically derived DNA probes, such as cDNA. The probes are generally attached in a two dimensional fashion onto a solid substrate and utilized in hybridization assays, in which they bind with complementary target sequences, derived from cell or tissue samples. In order to create the unique probe elements, full length oligonucleotides can be deposited in nanoliter-sized droplets to predefined positions on the microarray substrates using pin-spotting or inkjet printing techniques, or they can be assembled nucleotide by nucleotide directly on the substrate.

Microarray Substrates

Glass, which is a nonporous and relatively homogenous surface, is most often used as the microarray substrate material. Its low cost, availability, and relatively low inherent fluorescent background contribute to it being a favorable microarray substrate material. Another important property of glass is that it can be relatively easily modified through the use of alkoxy- or chlorosilanes, which can confer various properties to the glass; for example, silanes can alter the hydrophobicity or hydrophilicity of the glass or make the glass reactive to electrophillic or nucleophillic reagents. Consequently, particular silanes can be chosen to permit the immobilization of molecules of biological of interest, including oligonucleotides, to microarrays and other glass substrates.

Keysight Microarrays

Keysight Technologies, Inc. microarrays contain 60-meroligonucleotide probes that are synthesized in situ; meaning the probes are synthesized “on the fly” [Leiske 2006, Shen 2007]. Keysight’s non-contact SurePrintTM inkjet printing technology deposits extremely small (picoliter), accurate volumes of nucleotide monomers and ancillary reagents directly from sequence files to precise locations onto the microarray substrates, so that

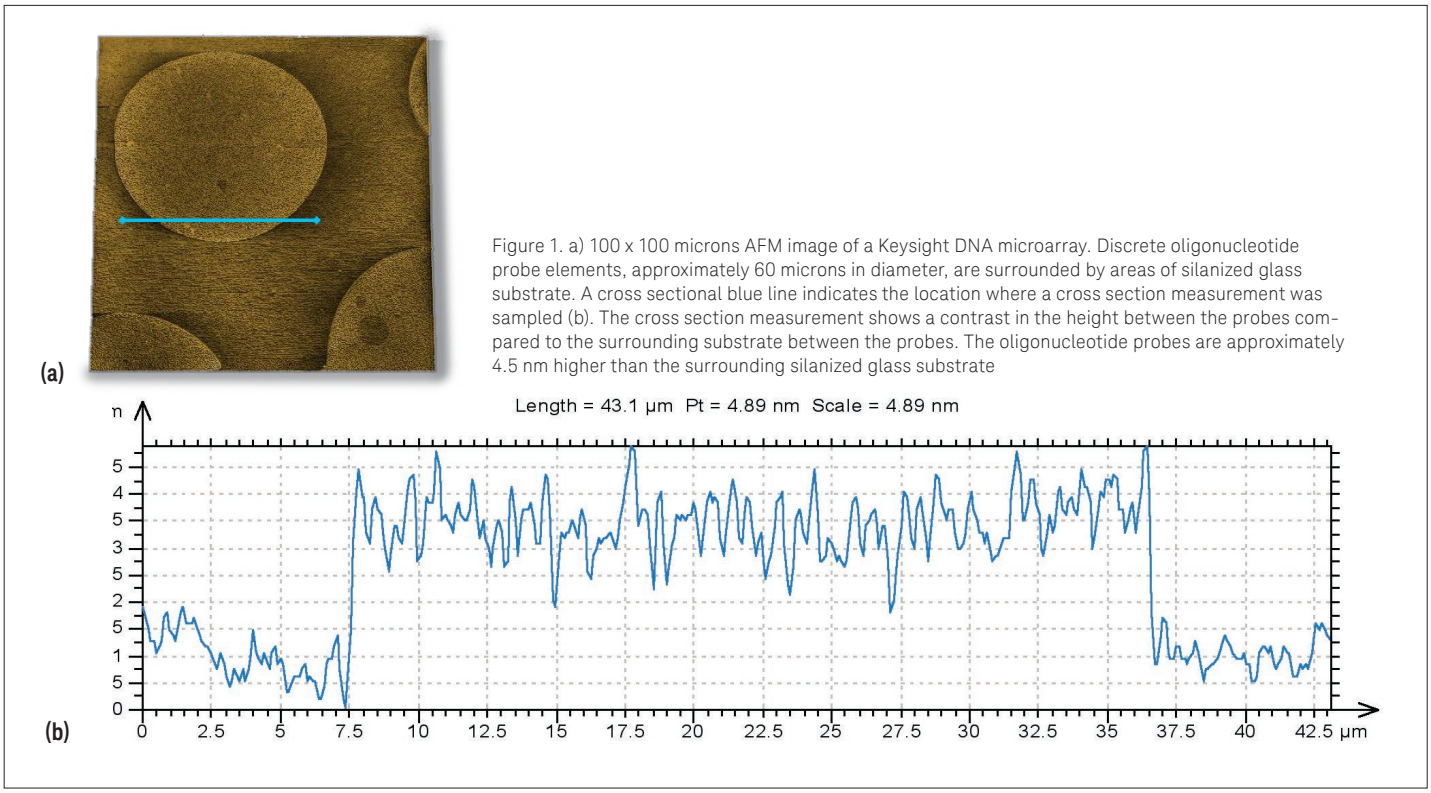

Figure 1. a) 100 x 100 microns AFM image of a Keysight DNA microarray. Discrete oligonucleotide probe elements, approximately 60 microns in diameter, are surrounded by areas of silanized glass substrate. A cross sectional blue line indicates the location where a cross section measurement was sampled (b). The cross section measurement shows a contrast in the height between the probes compared to the surrounding substrate between the probes. The oligonucleotide probes are approximately 4.5 nm higher than the surrounding silanized glass substrate

the probes are of high quality, extremely accurate and reproducible. Since the probes are assembled base-by-base, utilizing standard phosphoramidite chemistry, very high coupling efficiencies at each step are achieved, which ensures probe integrity and microarray quality.

AFM to Characterize Microarrays

Microarrays can offer challenges to their characterization and analysis due to the thinness of the materials applied over the underlying glass substrate, the similar refractive indices generated by the silanes of other materials on glass, as well as the small size of the discrete probe elements on the microarrays. Methods that can be used to characterize microarrays include blank microarray background fluorescence measurements, contact angle measurements and functional testing in biological assays. Contact angle measurement is a nondestructive technique that gives limited information about the presence or absence of materials on the glass. Background fluorescence measurements give only limited information about the inherent background fluorescence of the glass substrate but no information about the silane layers or the probe elements. Functional testing in controlled biological assays is a destructive method that yields critical information regarding probe uniformity, probe density, microarray performance and reproducibility [Peterson 2001].

There exist some other high resolution analytical tools, that can be used to characterize microarrays and which can be useful to help gain insight into microarray uniformity, quality and performance. These include microscopy and spectroscopy techniques, such as IR (infrared) spectroscopy, XPS (X-ray photoelectron spectroscopy), SEM (scanning electron microscopy, SPR (surface plasmon resonance), QCM (quartz crystal microbalance), and SAW (surface acoustic wave) [Berakdar 2004]. However, oligonucleotides are relatively small molecules, so the microarray probes are not easily visualized or measured by these conventional surface characterization techniques either. Plus, without the aid of extraneous labels, such as fluorescent dyes, microarray probes are often difficult to resolve against the background of silanized glass using spectroscopic techniques.

Figure 2. 70 x 70 microns 3D AFM image of the DNA microarray shows a single oligonucleotide probe element surrounded by the silanized glass substrate. The difference in height between the probe area and the bare substrate is clearly visible.

Figure 3. 15 x 15 microns 3D AFM image of the DNA microarray shows the contrast between a probe element and the surrounding silanized glass substrate (lower left).

Figure 4. At 3 x 3 microns, the border between the silanized glass (lower region) and the probe element (upper region) is clearly visible in the 3D FM image. Clumps of oligonucleotides in the probe area (upper right) can be seen rising above the substrate.

AFM is a powerful analytical, measurement and characterization tool that has been used to study a wide variety of inorganic, organic and biological materials and surfaces with nanoscale resolution. In particular, it has proven to be useful to image and characterize materials of biological importance without the use of extraneous labels. As demonstrated in this application note, AFM can be applied to the characterization of DNA microarrays; for example, to investigate the interface between the silane areas and probe elements, to measure surface roughness and give some indication of microarray probe spot uniformity and probe density.

AFM Measurements on Microarrays

As mentioned above, a typical DNA microarray contains thousands of unique oligonucleotide probes which are immobilized at discreet locations on silanized glass substrates. The manner in which probes are bound to the silanized substrate should be expected to impact microarray quality, reproducibility and assay performance. In this application note, AFM was used to characterize Keysight DNA microarrays. The AFM measurements were performed under ambient conditions using a Keysight 5500 AFM operating in acoustic AC (AAC) mode with a large Multipurpose scanner and PPP-NCH AFM probes (Nanosensors; Kc = 42 N/m, F = 330 kHz). The scan rate was 2 Hz and the scan range was varied from 100 x 100 microns to 1 x 1 microns. Cross sectional analysis was used to measure the variations in surface roughness and changes in thickness between the substrate silane layer and the immobilized oligonucleotide probes. A sharp contrast in height between the probe elements and the surrounding silanized glass substrate was observed; the probes are approximately 4.5 nm higher than the surrounding substrate.

In order to compare the surface roughness of the probe area to the silanized glass between the probes, the peak heights and RMS values of the surfaces were calculated. The peak height (or difference between the peaks and the plane) for the silanized glass substrate surrounding the probes was 2.34 nm with an RMS value of 0.429 nm, while the peak height for areas containing probes was 4.47 nm with an RMS value of 1.07 nm.

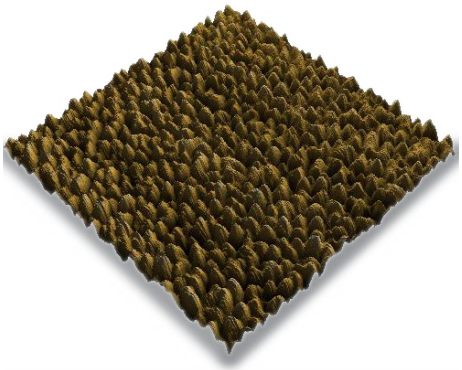

Figure 5. 1 x 1 micron 3D AFM image of oligonucleotide probes on the DNA microarray. The oligonucleotides appear to be clumped together in a uniform, evenly spaced manner. Using Pico Image, the probe area was measured to have a peak height of 4.47 nm and an RMS value of 1.07 nm.

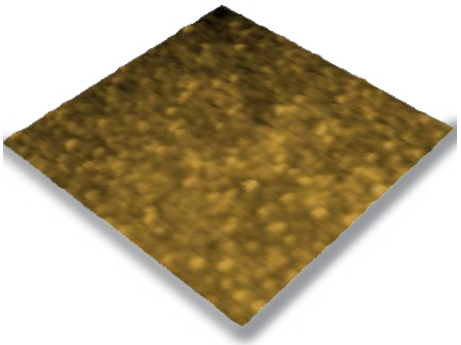

Figure 6. 1 x 1 micron 3D AFM image of the area between the probes (silanized glass substrate). There are no probe clumps visible in this area and, using Pico Image, the peak height in this area was measured to 2.34 nm; with an RMS value of 0.429 nm.

Conclusion

Keysight DNA microarrays, which are composed of 60-mer oligonucleotide probes that are immobilized on silanized, 1 x 3 inch glass substrates, were imaged by acoustic AC (AAC) mode AFM using a Keysight 5500 and a large Multipurpose AFM scanner under ambient conditions. The AFM images and cross sectional measurements indicate that, under ambient conditions, the probes are clumped together in a uniform manner, approximately 4.5 nm above the surrounding silanized glass substrate. AFM is particularly sensitive to surface defects and contamination, and it is expected that contamination might negatively contribute to DN microarray performance. As can be seen in the AFM images, almost no contamination was observed on the microarray; indicating that the Keysight microarrays are manufactured and packaged in extremely clean environments.

References

1. Berakdar 2004; Berakdar J, Kirschner J (2004) Correlation Spectroscopy of Surfaces, Thin Films, and Nanostructures; Wiley-VCH; 255 pages.
2. Conzone 2004; Conzone S and Pantano C Glass (2004) Slides to DNA Microarrays; Materials Today 7, 3; 20-26.
3. Heller 2002; Heller M (2002) DNA Microarray Technology: Devices, Systems, and Applications. Annual Review Of Biomedical Engineering 4; 129-153.
4. Leiske 2006; Leiske D, Karimpour-Fard, Hume P, Fairbanks B, Gill R (2006); Comparison of lternative 60-Mer Probe Designs In an In-Situ Synthesized Oligonucleotide Microarray. BMC Genomics 7; 72.
5. Peterson 2001; Peterson, Heaton R, Georgiadis R (2001) The Effect of Surface Probe Density On DN Hybridization; Nucleic cids Res 29; 5163-5168.
6. Shen 2007; Shen Y, Irons M, Miller D, Cheung S, Lip V, Sheng X, Tomaszewicz K, Shao H, Fang H, Tang H, Irons M, Walsh C, Platt O, Gusella J, Wu B (2007). Development of a Focused Oligonucleotide- rray Comparative Genomic Hybridization Chip for Clinical Diagnosis of Genomic Imbalance; Clin Chem 53, 12; 2051-2059.

AFM Instrumentation from Keysight Technologies

Keysight Technologies offers high-precision, modular AFM solutions for research, industry, and education. Exceptional worldwide support is provided by experienced application scientists and technical service personnel. Keysight's leading-edge R&D laboratories are dedicated to the timely introduction and optimization of innovative and easy-to-use AFM technologies.

www.keysight.com/find/AFM

For more information on Keysight Technologies' products, applications or services, please contact your local Keysight office. The complete list is available at: www.keysight.com/find/contactus

Americas

Canada	(877) 894 4414
Brazil	55 11 3351 7010
Mexico	001 800 254 2440
United States	(800) 829 4444

Asia Pacific

Australia	1 800 629 485
China	800 810 0189
Hong Kong	800 938 693
India	1 800 112 929
Japan	0120 (421) 345
Korea	080 769 0800
Malaysia	1 800 888 848
Singapore	1 800 375 8100
Taiwan	0800 047 866
Other AP Countries	(65) 6375 8100

Europe & Middle East

Austria	0800 001122
Belgium	0800 58580
Finland	0800 523252
France	0805 980333
Germany	0800 6270999
Ireland	1800 832700
Israel	1 809 343051
Italy	800 599100
Luxembourg	+32 800 58580
Netherlands	0800 0233200
Russia	8800 5009286
Spain	800 000154
Sweden	0200 882255
Switzerland	0800 805353 Opt. 1 (DE) Opt. 2 (FR) Opt. 3 (IT)
United Kingdom	0800 0260637

For other unlisted countries:
www.keysight.com/find/contactus
(BP-09-23-14)

KEYSIGHT TECHNOLOGIES

Published in USA, July 31, 2014
5989-9946EN
www.keysight.com