In-situ synthesis and direct immobilization of DNA oligonucleotides on pre-patterned HSQ nanostructures

*Omar D. Negrete**, M. Serdar Onses[#], Paul F. Nealey[#], and Franco Cerrina**
**Dept. of Electrical and Computer Engineering, University of Wisconsin-Madison*
[#]Dept. of Chemical and Biological Engineering, University of Wisconsin-Madison
1514 Engineering Dr., Madison WI. 53706 USA

A necessary step in the sequence specific localization of DNA nanostructures and biomolecules is the patterning of ssDNA on surfaces. Hence, we have studied the ability to localize the synthesis and immobilization of ssDNA through the use of pre-patterned Hydrogen Silsesquioxane (HSQ), providing a method for the fabrication of nanoscale ssDNA spots, limited only by the resolution of the HSQ; a well-known high-resolution negative tone resist. After e-beam exposure or thermal curing HSQ densifies, and becomes "glass-like" with properties that are expected to be suitable for bio-chip applications[1].

For this demonstration, HSQ was patterned with an e-beam writing system to fabricate HSQ arrays ranging in spot size from 1μm to 50nm on both chrome and native oxide surfaces. To show that e-beam patterned HSQ can serve as a solid support for light-directed synthesis of ssDNA, NPPOC-protected phophoramidite photochemistry and a maskless UV exposure tool[2] were employed to synthesize DNA onto the HSQ arrays. As a complementary technique, immobilization of pre-synthesized ssDNA using a covalent coupling strategy involving a silane (APTES), homofunctional linker (PDITC) and end-modified (amine) oligonucleotide, was performed[3]. To measure the selectivity and efficiency of HSQ-bound ssDNA, complementary and non-complementary Cy-3 labeled oligonucleotides were hybridized and measured for fluorescence (Figs. 1 and 2).

Nano-scale arrays greater than 50nm were identifiable by fluorescence microscopy. This demonstrates that e-beam patterned HSQ can be functionalized with ssDNA and subsequently used as sequence specific nanoscale probes. Additionally, background noise due to non-specific binding on to substrates was observed and this may inhibit the specificity of these DNA nanoarrays. As a result materials that can passivate the background surface of HSQ arrays against non-specific binding is being further investigated. The use of patterned HSQ in this manner offers new opportunities in bio-chip applications that require high-sensitivity, reduced reagent cost, and for integration into lab-on-chip devices.

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[1] S. Choi, M.J. Word, V. Kumar, and I. Adesida, J. Vac. Sci. Technol. B, **26**, 5, 2008.
[2] S. Singh-Gasson, R.D.Green, Y.Yue, C.Nelson, F.Blattner, M.R.Sussman, F.Cerrina, Nat.Biotech.,**17**, 1999.
[3] Z. Gou, R.A. Guilfoyle, A.J. Thiel, R. Wang, and L.M. Smith, Nucleic Acids Research, **22**, 24, 1994.

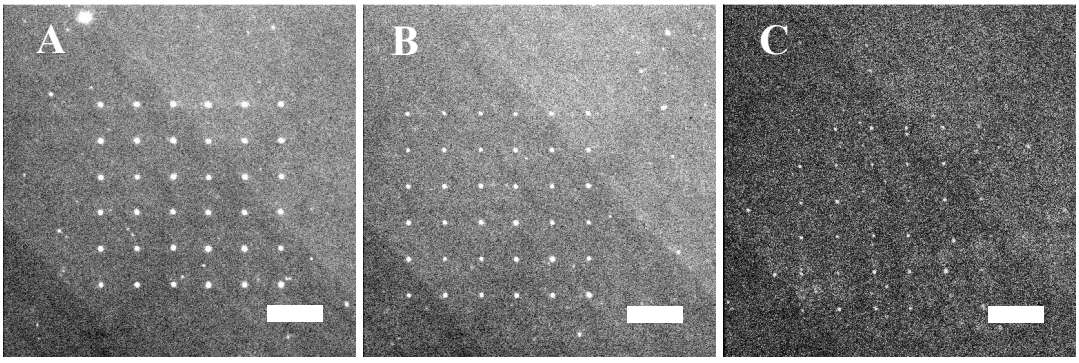

Figure 1: In-situ synthesized DNA on 6x6 HSQ arrays on native Si oxide substrate. Fluorescent signal from hybridization with Cy-3 labeled complementary sequence: (A) 500nm (B) 250nm and (C) 100nm HSQ arrays. Features below 100nm were not clearly observed by fluorescence. Array pitch is 13.7µm. Images taken with a Nikon E800 microscope, Metamorph image software, scale bar = 20µm.

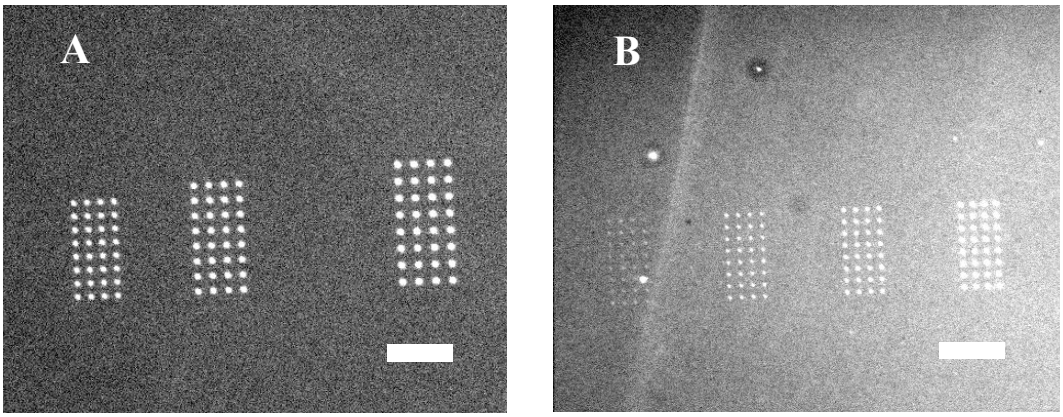

Figure 2: Direct immobilized DNA on 4x8 HSQ arrays on native Si oxide substrate. Fluorescent signal from hybridization with Cy-3 labeled complementary sequence: (A) 800 nm, 900 nm and 1 µm (From left to right) (B) 100 nm, 150 nm, 200 nm and 300 nm HSQ arrays. Array pitch is ~3.0 µm. Images taken with a Nikon E800 microscope, Metamorph image software, scale bar = 20µm.