SANDIA REPORT

Highly Specific Electronic Signal Transduction Mediated by DNA/metal Self-Assembly

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Abstract

Highly specific interactions between DNA could potentially be amplified if the DNA interactions were utilized to assemble large scale parts. Fluidic assembly of microsystem parts has the potential for rapid and accurate placement of otherwise difficult to handle pieces. Ideally, each part would have a different chemical interaction that allowed it to interact with the substrate only in specific areas. One easy way to obtain a multiple chemical permutations is to use synthetic DNA oligomers. Si parts were prepared using silicon-on-insulator technology microfabrication techniques. Several surface chemistry protocols were developed to react commercial oligonucleotides to the parts. However, no obvious assembly was achieved. It was thought that small defects on the surface did not allow the microparts to be in close enough proximity for DNA hybridization, and this was, in part, confirmed by interferometry. To assist in the hybridization, plastic, pliable parts were manufactured and a new chemistry was developed. However, assembly was still absent even with the application of force. It is presently thought that one of three mechanisms is preventing the assembly. The surfaces of the two solid substrates can not get in close enough proximity, the surface chemistry lacks sufficient density to keep the parts from separating, or DNA interactions in close proximity on solid substrates are forbidden. These possibilities are discussed in detail.

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I. INTRODUCTION

DNA/DNA interactions allow for a large number of designed permutations. In addition, the commercial availability of DNA and the vast literature make DNA an attractive target for applications with high permutation interests. However, one problem with common DNA publications is that extracting information about where and how successful the hybridzations were is typically done using signal transductions mechanisms such as fluorescence. If DNA can be tagged to things that more easily render themselves detectable, then extracting the high permutation count from DNA interactions would be significantly easier.

There are a variety of technological advantages to Microsystems. However, by nature, Microsystems suffer from any processes for which only macroscopic techniques have been developed. A good example is the assembly of solid piece parts. While it is rather trivial to produce a variety of materials with tens of microns on dimension and sub micron tolerances, if the pieces are free standing at the end of the process, pick and place assembly techniques need to replicate the process tolerance of the lithographic sequence, and this is a particularly challenging task. Other potential techniques such as electrophoretic deposition have similar problems; aligning the assembled part to the tolerance of modern lithography is a difficult challenge. Because of this, multi-level assemblies and fabrications processes, for which dissolution of 77sacrificial layers occurs, have been almost solely relied upon to solve the assembly issues.

One potential technique is to pattern the final substrate and use fluidic self-assembly to assemble the parts. In this way, the tolerance and registration of the parts can be defined by the lithographic patterning of the substrate if the fluid process is of sufficient fidelity. Another advantage is that it is typically inexpensive to produce single layer piece parts in extremely high numbers. The efficiency of the fluidic assembly does not need to be particularly high, only the fidelity of registration. Fluidic assembly of parts has been demonstrated for some time.(Yeh and Smith 1994; Gracias, Tien et al. 2000; Srinivasan, Liepmann et al. 2001) However, in the technique developed by Smith, the parts were shape-fitted to the substrate. This technique has been utilized in very high volume, however, and shows the potential of the fluidic assembly technology. One advantage of Smith's method is that various shaped parts can be assembled onto the substrate. If the parts assemble only into wells of their respective size, then the largest parts can be exposed to the substrate and will not interact with the smaller holes. Similarly squares will not fit into round holes, etc. Sequentially, then, one can build up an array of different structures on the same substrate. The number of different parts determined by the number of assembly steps that can be tolerated.

Fluidic assembly has also been shown for certain chemical interaction based on surface tension forces.(Gracias, Tien et al. 2000; Srinivasan, Liepmann et al. 2001) This is a particularly intriguing technique because it is not required that the final part be fixed into place. In addition, if the parts need to be fixed into place they can be, and they can even be electrically addressed. Srinivasan et al. have shown sub micron registration accuracy using this method. One major problem, however, is that the parts cannot discriminate shape on the surface, only surface tension. As a result, a hydrophobic circle on the substrate may capture a hydrophobic square part of larger or smaller size when assembled in water. As such, one would have to do sequential lithography of the surface, then assembly, then lithography and assembly, to build up systems

with different types of parts. This may be efficient in some circumstances, but clearly has limitations.

A need exists, then, to tag each type of parts with a different chemical species and to pattern the substrate with the required complementary species to each part. It would also be ideal of species A on part A could not interact with complement B on the substrate. That is, the lithography of the substrate determined only the registration and alignment of the parts, but the tagging of the parts determined where they go. One way to do this is to utilized DNA interactions. Synthetic DNA analogues are commercially available and can be tailored to interact only with their complementary strand under the correct circumstances. A 20 base pair oligomer has 4^{20} permutations, giving the ability to tag many different types of parts simultaneously. In addition, photolithographic patterning of hundreds of thousands of known, specific DNA strands onto surfaces is a mature field. The force of a single DNA strand hybridized to its exact complement has been measured to about 70pN (Wenner, Williams et al. 2002). A typical DNA surface density is approximately 10^{12} molecules/cm^2. This should allow for at approximately 70 N of force per cm^2 in tension for two, DNA-attached solid surfaces hybridized together. For a 10 μm thick part the force vs. gravity would be approximately 20 μN(density 2.2 g/cm^3), showing that even moderate surface densities of DNA should be able to support the mass of the piece parts in pure tension. In peel, the force needed to break the DNA bonds would be considerably less, but given that there is about 6 orders of magnitude greater potential adhesive force available, it would seem as if DNA assembly should be possible. Hence, the ability to assemble a nearly infinite number of types of parts simultaneously exists if DNA-DNA, solid-solid interactions can be shown to occur and to be specific.

II. EXPERIMENTAL

A. Silicon Parts: The process flow for Si parts is shown in Figure 1a-e. Silicon-on-insulator (SOI) wafers were purchased from Ultrasil, Inc. (Hayward, CA). The wafers were (100) orientation with device thickness of approximately 7.5 +/- 2.5 μm thickness, and an oxide thickness of approximately 1.5 +/- 0.5 μm. 7.5-8 μm of SJR 5740 (Shipley) photoresist was applied to the SOI wafer and patterned using a proximity/contact aligner and standard development recipes. A deep reactive ion etched was then performed at 20°C chuck temp and 10 Torr He. Etch A was 2 sec. long, with 8 W RF power, 850 W Inductively Coupled Plasma (ICP) and Ar of 40 sccm, and SF_6 at 50 sccm. Etch B was 7 sec. long, 8 W RF power, 850 ICP, 40 sccm Ar and 100 sccm SF_6. Deposition C was 5 sec. long, 1W RF, 850 ICP, 40 sccm Ar, and 70 sccm C_4F_8. This process was repeated approximately 200 times to give a total etch depth of 32 μm. The process was scaled to etch the device layer thickness. The wafer was then soaked in Acetone to remove the resist and exposed to hot piranha solution to clean the residue. Finally, a concentrated HF (49%) dip for approximately 15 min. was sufficient to release the parts from the substrate. The parts were filtered through a standard, Whatman filter, rinsed, and suspended in methanol for surface derivatization. From the remaining parts on the wafer, the yield of parts floated appeared to be no more than about 10% but was sufficient to yield experimental material. SU-8 photoresist and was purchased from MicroChem Corp. (Newton, MA).

B. Surface Chemistry. Initial surface chemistry was performed in 1.5 ml, plastic vials. Stirring was done via vortexing, and washing was through centrifuge and decant. It was shown early that this process was unlikely to create pure enough samples. The second procedure was done in solid phase synthesis glassware purchased from Peptides International. Oligonucleotides were purchased from Trilink Biotechnologies and were purified using HPLC and are shown in Figure 2. Polydisperse polyethylene glycol (PEG) heterobifunctional linkers were purchased from Shearwater Polymers (Hunstville, AL,) with a nominal weight average molecular weight of 3400 g/mol, **4** in Fig. 2. Discrete PEG crosslinkers were purchased from Quanta Biodesign (Powell, OH). **5** in Fig.2. Amino propyl methoxy dimethyl silane was purchased from Acros Organics. All other reagents were purchased from Aldrich.

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III.   RESULTS AND DISCUSSION

A. Initial Handling and Experiments: Silicon piece parts were fabricated as per Figure 1. The parts were nominally 200 μm in "diameter" and consisted of circles (cylinders in three dimensions), squares and "snowcones". The snowcones possessed only a single mirror plane and no rotational symmetry so that "clocking" of assembly could be evaluated. Initially, the parts were subjected to epoxypropyl trimethoxy silane in xylenes for 1 hr. at approximately 80°C, followed by reaction with the 5' aminated version of **1** for approx. 24 hrs. at room temperature. The parts were then hybridized with **3** and imaged with confocal microscopy. It was clear from observations during the synthesis procedure that obtaining uniform surface coverage on the parts was a difficult task. First, the parts were typically reacted within 1.5 ml plastic vials and shaken vigorously. However, if the wetting of the parts by the reacting medium was poor, then the parts clearly stuck together or tended to float on top of the reacting medium. In addition, ultrasonication of the parts was found shatter them rather efficiently and could not be utilized for mixing. It was also found to be rather difficult to purify the reacting medium. In this experiment, centrifuge and decant was used. The yield in handling the parts and transferring proved to be rather poor. In Figure 3, one can see a fluorescence image of a circular part. It appears rather non-uniform in fluorescence intensity. This could be because of the ability to disperse the parts evenly within the fluid so that an even reaction could occur. Because of the observations of problems from the surface chemistry and the fluorescence image, it was concluded that proper handling and reacting to the microparts was critical to the success. However, Fig. 3 does show that tagging the microparts can be done with DNA.

In order to assess the issues involved with the proper surface chemistry in aqueous media, and to practice with the new techniques, an experiment was devised to simply coat the parts and glass slides with hydrophobic and hydrophilic coatings and learn from simple assembly. In addition, the use of the peptides glassware allowed filtering and rinsing at least 6 times between chemical exchanges, a critical cleaning improvement necessary for the low moles of reagent needed for surface chemistry. The aminated parts were then reacted with palmityl N-hydroxysuccinimde ester in dissolved in DMF then brought to 6 mM in the presence of 15 mM $NaHCO_3$. After reaction, the parts were placed in water. It was clear that the parts were extremely hydrophobic. The hydrophilic parts were cleaned in Nanostrip at greater than 60°C for 2 hours as shown in Figure 4. The hydrophilic and hydrophobic glass slides were treated with the exact same chemical sequences. A number of piece parts was removed and pippetted onto the glass slide for observation. The water was allowed to dry and then rinsed to determine whether the parts could be removed from the surface. The first observation that was apparent was that the hydrophobic parts were often stacked and agglomerated. This makes some sense as the surface of the parts was not wetted by the fluid medium. However, this confirms the result from Fig. 3 that it may not be possible to achieve good, uniform surface chemistry unless the parts are wet by the reacting medium. The second observation from this experiment is shown in Figure 5. On the left side are the as-deposited piece parts on substrates. For clarity, only the extreme cases of hydrophobic/hydrophobic and hydrophilic/hydrophilic are shown. On the right side is after several washings in deionized water. One can clearly see that the hydrophilic parts can be removed by washing with deionized water while the hydrophobic parts cannot. This has two implications. First, the size and mass of these parts means that surface wetting/chemistry can

dominate the physical behavior. It will be imperative for proper surface chemistry and mixing of the parts to occur that the reacting medium and the parts are always compatible in terms of wetting. Second, the density of the parts is greater than 1 and they tend to sink rather readily in solvents. One possible technique for assembly will be to have an excess of parts placed over the surface. The parts not assembling in proper sites can then be removed by simply washing with a high density liquid such as sodium tungstate in water or perfluorodecalin, so that they float away. Exposure of non-wetting parts to high density fluids did not assist in their removal from the substrate. The control of wetting is critical in the surface chemistry as well as the assembly process.

B: Surface chemistry modifications. The prior section clearly showed the necessity for properly designed surface chemistry. The next attempt was to design a surface preparation that was reliable chemistry (there is little opportunity for chemical analysis after each step on the piece parts, but this is described below), yet all of the reacting fluids wet the parts nicely during the reaction.

The next surface chemistry scheme is shown in Figure 6. Typically, approximately 1/8 surface area of a 4 in. wafer of parts was used in approximately 2 ml of reacting fluid. Because of the tendency for trimethoxy silanes to polymerize and have poor process latitude, a monomethoxy silane was used to aminate the surface. Aminopropyl, methoxydimethyl silane was used and it was shown that the 90/10 mixture of isopropanol and water wet the reacted, aminated silicon surface as well as chloroform. Chloroform dissolves the heterobifunctional PEG and also wets the aminated silica. For all of these steps, vigorous shaking and mixing via a vortexer and an aggressive paint shaker was used to ensure mass transfer of the reactant to the particulate surfaces. In addition, it was shown via NMR that the NHS ester on the PEG in step 2 was stable in dry chloroform for at least 4 hrs. After each step, at least 6 rinses were made in the reacting vessel to remove traces of the prior reactant so that the subsequent reactants will react with the surface. It was clear visually from this procedure that vast improvements for wetting of the solvent and reactants with the parts was achieved. The resulting parts were hydrophilic, but not as hydrophilic as the received, hot-nanostrip-cleaned parts.

Assembly was attempted in several different configurations. Slow mixing for 4 days with a glass slide reacted with the complementary strand was attempted. Also, parts of two different shapes (each with a complementary strand) were mixed together for days. No obvious agglomeration between parts with complementary strands was achieved.

It was thought that possibly the density of DNA on the surface was poor because of the use of the long PEG chain in step 2 of Figure 6. To alleviate this, and to greatly increase the concentration, a discrete PEG was chosen from Quanta biodesign. The resulting parts appeared to be more hydrophilic with the discrete PEG. Not only does the molar concentration of discrete PEG increase by approximately a factor of 8 in the reacting medium given the 100 mg aliquots available, but also the smaller size of the PEG should allow for higher packing on the surface. One observation was that after the discrete PEG synthesis, the parts appeared more hydrophilic, a hopeful indication that the surface density of DNA was considerably higher. However, several attempts at hybridizing two different parts were still unsuccessful.

Despite the lack of good analytical work after each step of the synthesis, it was thought that the improvements in mixing, wetting and reaction were yielding parts with DNA attached. However, no hybridization was observed. Next, It was thought that the parts were potentially not flat. It is typical for 4 in. wafers to exceed 10 μm of bow. Since the characteristic length of a part divided by its thickness is approximately that of a typical 4 in. wafer of 500 μm thickness, we extrapolate linearly and this gives approximately 20 nm of bow across a 200 μm part. Because the resultant thickness of surface chemistry is likely to be less than 10 nm, it is possible that the bow in the parts is not allowing for hybridization. To address this, some parts were pipetted onto a glass slide for interferometric measurements. Figure 7 shows a typical result from the initial measurements. Particulate matter extends several hundred nm from the part and the whole part is skewed vs. the substrate by a similar amount. This indicates that dust may be keeping the solid parts from achieving close enough contact for hybridization. Figure 8 shows the second interesting result. The parts do not appear to have appreciable bow, less than approximately 20 nm. Surprisingly, bow does not appear to be a problem, even though particulate matter does. A quick calculation brings this to focus. Typical dirty wafers can have at least 10,000 particles/cm^2. Since a square part has 4×10^{-04} cm^2, an average part would have as many as 4 particles on it. A considerable amount of care must be taken to insure that parts do not have particulate matter on them. From the extremely brief interferometry measurements, it was clear that some of the parts did not appear to have particles on them, while some did. However, even a 50 nm particle could cause problems for the stiff, silicon substrates to get solid phase hybridization.

C: Plastic Parts. While the surface chemistry appeared to be improved, it was clear that particulates on inflexible piece parts may be problematic toward hybridization. It was not considered practical in the present scheme to remove all possibility of particulate matter in the synthetic procedure. One suggestion, then, would be to work with much smaller, stiffer parts, on the order of 20 μm in characteristic length with 5 μm thickness. Another suggestion would be to utilize flexible parts. A scheme was devised to make solid phase, flexible parts in an SOI-type fashion. SU-8 photoresist was utilized. First, the high density of epoxide groups in the resist means that the final resist is not fully reacted, leaving the potential for facile, robust, epoxide coupling to the surface of the parts. Second, SU-8 is easily flexiblized with the addition of reactive diluents.

Commercial SU-8 photoresist was flexibilized using 20 wt.% (to solids) of polyethylene glycol diglycidyl ether, M_n 380 g/mol. The resulting resist was spun and had a post apply bake of 90°C, 8 min. and an exposure of 1000mW/cm^2. The post-exposure bake was 90°C for 9 min and development was in PGMEA for 2x the time to clear unexposed regions. Piece parts made from this method had no cracking and withstood more than a 1 month soak in acetone and 1,1,1, trichloroethane with no visible degradation. To make parts, then, the resist was spun onto a 0.5 μm thick wet oxide on a Si wafer, and after lithography 20 min. of conc. HF was utilized. The parts did not float easily, but ultrasonication for a few minutes resulted in nearly complete release from the substrate without any damage to the plastic parts.

To tag the parts, the parts were suspended within a 4.4 wt.% solution of DAB-AM-64, polyamine dendrimer in ethanol for approximately 1 hr. at 70°C and then 10 days at room temperature. Next, 100 mg of the discrete PEG in dry chloroform with 0.15 M triethyl amine

was shaken for 4 hrs. Then, the 5'thiolated DNA **1** was exposed to the free maleimide. Unfortunately, no assembly between complementary tagged parts resulted. Because of the flexibility of the plastic parts, it was possible to put circles and squares with complementary strands on them onto the same glass slide and apply force to a stack with a circle and square. Even the application of force was unable to get the parts to stick.

D: Future work/suggestions. Oligonucleotide specific hybridization between two solid parts was not accomplished. At this point, there are three prevailing theories as to why this could be. First, the surface chemistry used was not yet robust enough. Second, the physical constraints of two solid surfaces does not provide the ability to hybridize, and the third is that DNA-DNA solid phase hybridization is forbidden.

To address the surface chemistry issues, it is clear that surface analytical techniques will be needed. Despite solution phase robustness, surface chemistry is different in that mass transfer to the surface significantly limits achieving high density. Also, high density surface chemistry is extremely difficult due to steric hindrance from previously reacted species. One way to analyze the surface concentration of species on the piece parts is to quantify the amine content using a known assay. For example, after the step involving the silane coupling, a trityl protected sulfosuccinimide ester such as SulfoSDTB from Pierce can be reacted to the parts. Mild acid cleavage of the trityl ion produces an extremely absorbing species that can be measured with known absorbance using UV-Vis even at surface concentrations. In this way, the amine content on the as-reacted piece parts can be quantified. Quantifying the reaction of the PEG can be done similarly. One would need a t-Boc protected amine on one end of a PEG and an NHS ester on the other. These are available from Shearwater and Quanta biodesign. In this scheme, the NHS ester of the PEG is reacted to the amine on the surface. An acid is then used to expose the amine on the other end of the PEG. The amine assay is then performed to quantify the amount of amine present. It may be necessary in such case to use an NHS ester short chain to cap all of the free amine from the Si surface. However, in this way, one can quantify the amount of PEG placed on the surface with a similar molecular weight PEG. Quantifying the DNA is a bit more difficult. However, radiolabelled DNA can be purchased from several companies to quantify the amount of DNA put onto the maleimide end of the PEG. In this way, one can quantify the amount of DNA present on the surface and also optimize the various intermediate chemistry steps.

To get the surface density of species even higher, and to extend the DNA oligomers further from the surface, some surface polymerizations may be needed. It is possible, however, to extent the free DNA oligomers greater than 10 nm from the surface and to allow them the requisite flexibility to hybridize.

The second possibility that is interfering with the assembly might be physical occlusion. Figure 7 clearly shows that particulate matter can get in the way of potential hybridization. However, it is not particularly difficult to achieve particulate densities lower than 10,000/cm^2 using standard clean room techniques and cleanroom grade solvents. In addition, one could decrease the part size to below 20 micron so that the probability of a particulate (given 10,000/cm^2) falls below 4% and this problem may be subverted. However, the surface roughness of typical solids easily can exceed 10 nm. Bringing two solid surfaces together at the scale necessary to achieve

hybridization may require that the surface chemistry achieve flexible interacting groups at lengths greater than 10 nm. One simple suggestion to achieve this is to begin the work using streptavidin coated substrates and tag the parts with biotin. While there does not exist the ability to scale up permutations using this scheme, the streptavidin-biotin bond is extremely strong and robust. Biotin-labeled PEGs are common, and this interaction would allow one to quickly quantify the issues associated with physical mismatches such as getting round surface debris and surface roughness.

The last possibility is that constrained DNA/DNA interactions are difficult to achieve. Mirkin and co-workers have shown nanoparticle networks using DNA interactions, but not flat surfaces.(Mucic, Storhoff et al. 1998; Jin, Wu et al. 2003) The distinction here is the effective concentration of salt and DNA when subject to a constrained, 20 nm like dimension. Mirkin has shown that the melting behavior of DNA in the nanoparticle networks is to some extent different than solution DNA because of the high salt and DNA concentration between the networked nanoparticles. The problem will be amplified with flat, solid/solid surfaces. In addition, measurements on the force needed to pull DNA from its complementary strand quoted here have been for individual strands only. It is possible that cooperative interaction between the strands would not allow simple, linear extrapolation of the force to break one complementary strand to that of high surface density.

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IV. ACKNOWLEDGEMENTS

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V. REFERENCES

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Srinivasan, U., D. Liepmann, et al. (2001). "microstructure to Substrate Self-Assembly Using Capillary Forces." Journal of Microelectromechanical Systems **10**(1): 17-24.

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Yeh, H.-J. J. and J. S. Smith (1994). "Fluidic self-assembly of GaAs microstructures on Si substrates." Sensors and Materials **6**(6): 319-32.

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VI. FIGURES
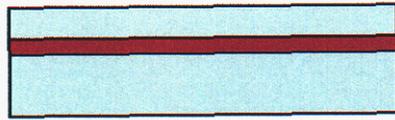
a. SOI wafer
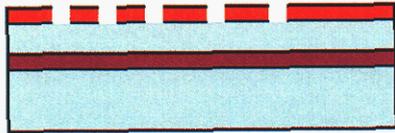
b. Apply and pattern photoresist
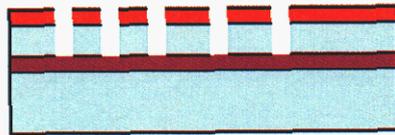
c. Dry etch through Si "handle" to oxide etch stop

d. Clean resist from wafer

e. Release parts with selective oxide etch and Filtration.

Figure 1 a-e: Processing sequence for Si parts. See text for conditions.

1   5'-HS (C6) GAGAAGAGAGCGAAGGAGA-3'

2   5'-HS (C6) TCTCCTTCGCTTCTCTTCTC-3'

3   5'-Fluorescein (C6)- GAGAAGAGAGCGAAGGAGA-3'
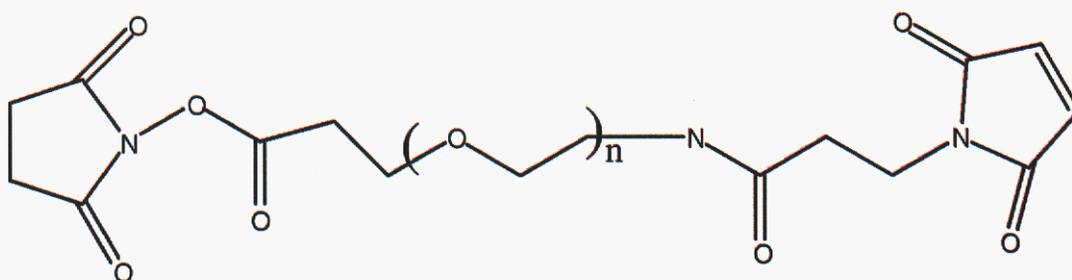
4 n avg. = 75, avg. m.w. = 3400

5 n = 4

Figure 2: Chemicals used in the tagging of parts.
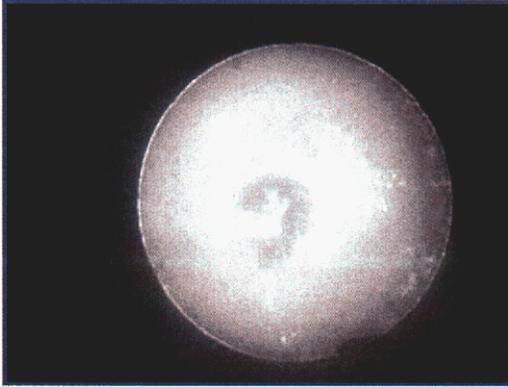
Figure 3: Fluorescence image of circular Si part with DNA **1** covalently bonded to the surface and DNA **3** hybridized to it.

Si piece part approx. 200x200x5	$+$	H_2SO_4/H_2O_2 $\longrightarrow$	Hydrophilic Surface

Si piece part approx. 200x200x5 $+$ [structure] $\longrightarrow$ Amine terminated surface

Amine terminated surface $+$ [structure] $\longrightarrow$ Hydrophobic Surface
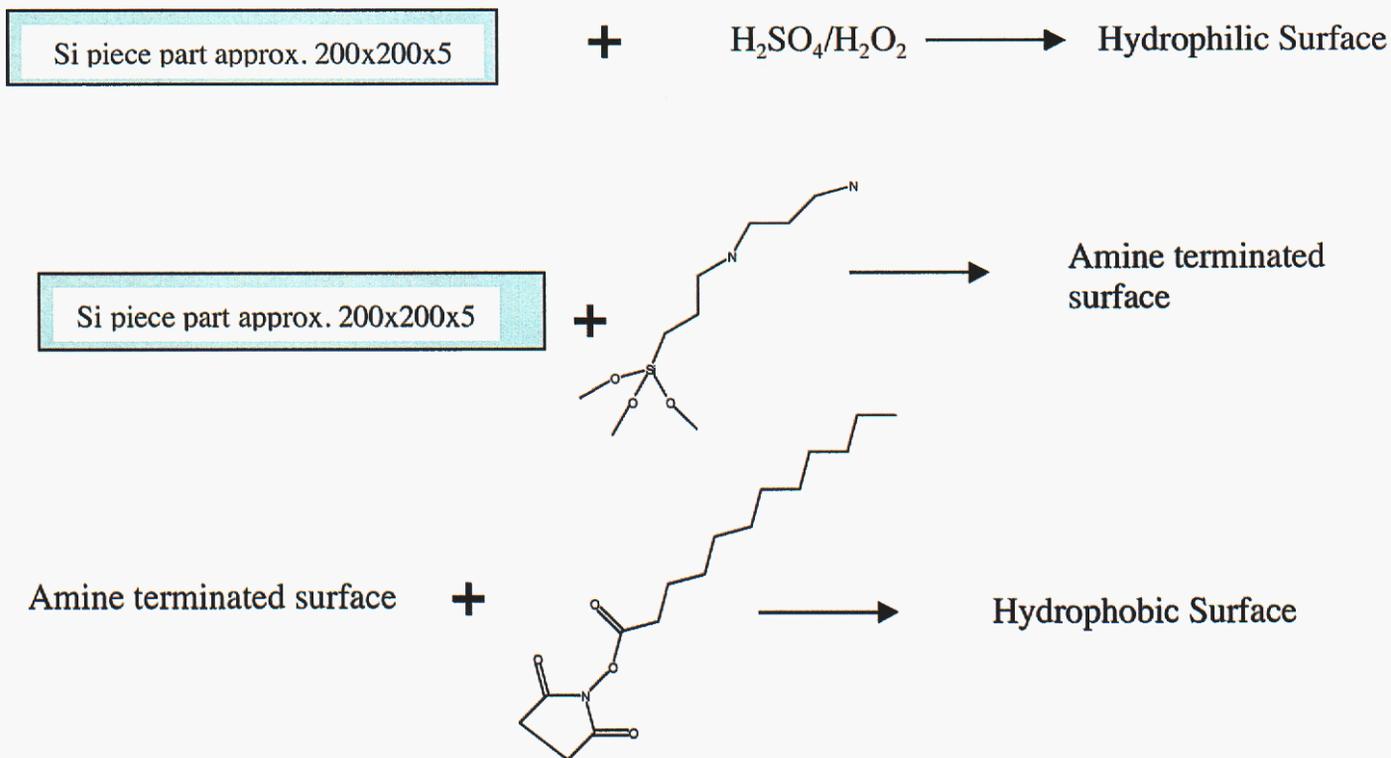
Figure 4: Chemistries resulting in hydrophobic and hydrophilic silicon surfaces. Parts were prepared with both the hydrophilic and hydrophobic treatments and surfaces were prepared with both the hydrophobic and hydrophilic treatments.
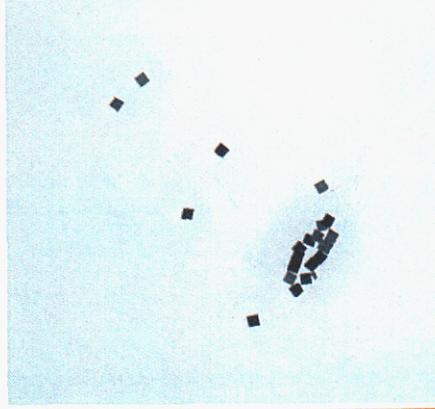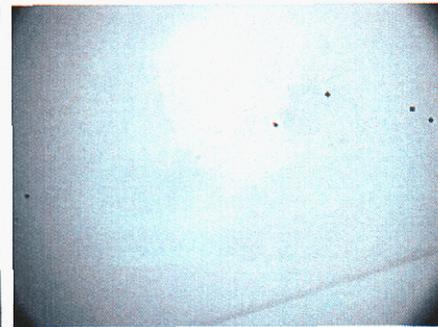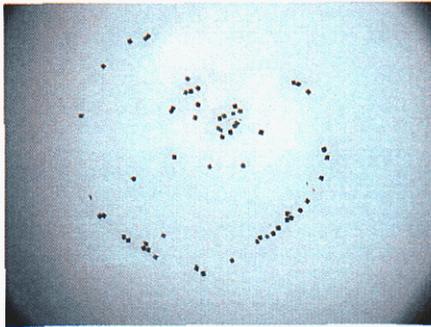
Figure 5: Si parts coated with hydrophobic and hydrophilic monolayers and substrates coated similarly. Hydrophobic parts are not washed from a hydrophobic surface with water. Similarly, hydrophobic parts showed an affinity to agglomerate.
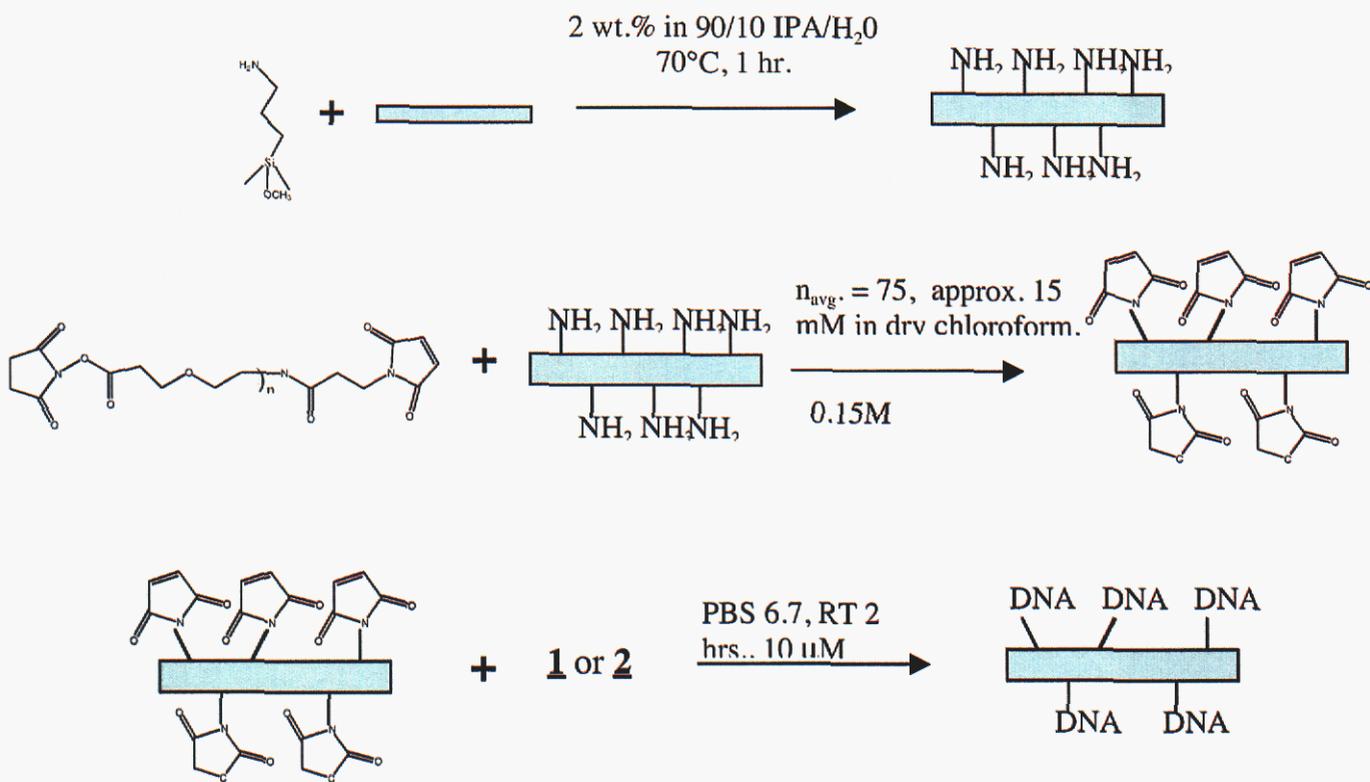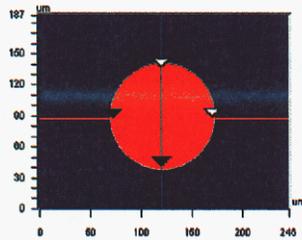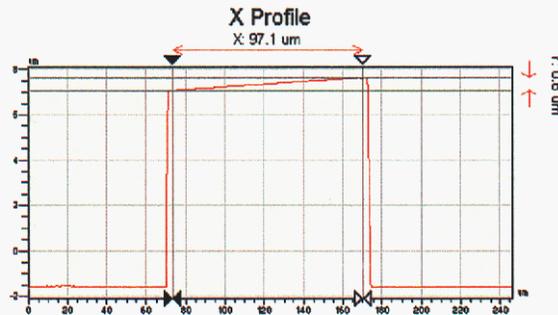
Figure 6: Surface chemistry scheme to keep the parts wetted by the reacting fluid. Several iterations were made to the reactant concentrations and are discussed in the text.

Figure 7: Interferometric data for a 200 μm circle. Top shows the title of the circle relative to the flat, glass substrate as approximately 0.2-0.6 μm. Bottom shows several surface asperites and indents. The asperites measure more than 0.2 μm.

X Profile
X: 90.6 um

Rq	0.00 um
Ra	0.00 um
Rt	0.02 um
Rp	0.01 um
Rv	-0.01 um

Angle	-238.29 urad
Curve	1.48 m
Terms	None
Avg Ht	-0.00 um
Area	-0.11 um2

Y: 0.022 um

Y Profile
X: 70.5 um

Rq	0.01 um
Ra	0.01 um
Rt	0.05 um
Rp	0.01 um
Rv	-0.04 um

Angle	-186.14 urad
Curve	0.20 m
Terms	None
Avg Ht	-0.00 um
Area	-0.31 um2

Y: 0.013 um

X	53.51	-	um
Y	50.73	-	um
Ht	-2.30	-	nm
Dist		-	um
Angle		-	°

Title:

Note:

Mag: 103.1 X
Mode: VSI

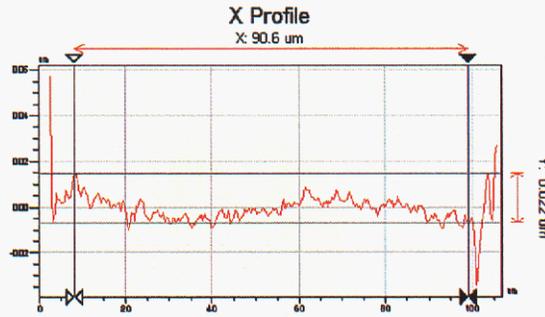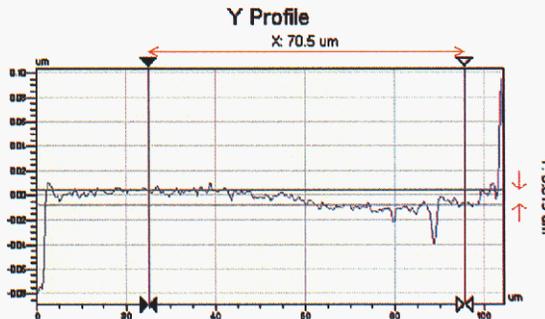**Surface Data**

Date: 05/28/2003
Time: 12:36:30

Surface Statistics:

Ra: 11.65 nm
Rq: 24.15 nm
Rz: 489.59 nm
Rt: 682.30 nm

Set-up Parameters:
Size: 736 X 480
Sampling: 81.47 nm

Processed Options:
Terms Removed
Tilt
Filtering:
None
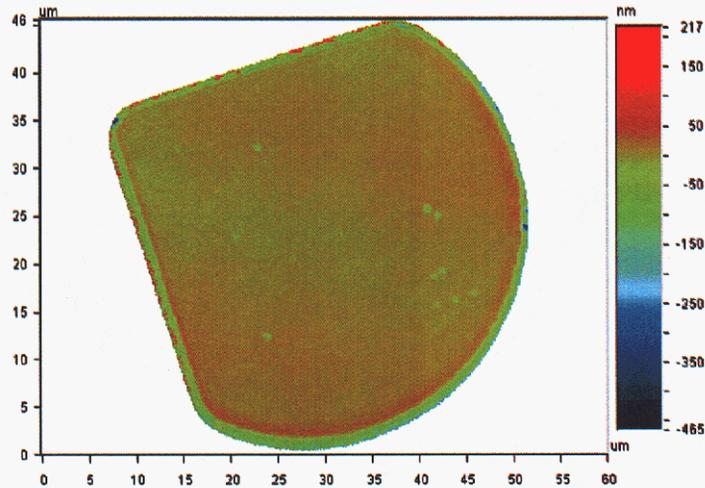
Title:

Note:

Figure 8: Interferometric measurements on a snowcone indicating "potato chip" effect. The "warpage" is only a few tens of nm peak to peak across the part, but the bottom view again shows particulate matter capable of precluding DNA hybridization.

VII. DISTRIBUTION

1	MS-FJ39	Jim Schicker
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