Photoelectrochemical Array Platform for
Genomic Scale DNA Synthesis

by
Christopher Joseph Emig

Submitted to the Department of Electrical Engineering and Computer Science
in Partial Fulfillment of the Requirements for the Degree of
Master of Engineering in Electrical Engineering and Computer Science
at the Massachusetts Institute of Technology
February 13, 2006

Copyright 2006 Massachusetts Institute of Technology. All rights reserved.
Photoelectrochemical Array Platform for
Genomic Scale DNA Synthesis
by
Christopher Joseph Emig

Submitted to the Department of Electrical Engineering and Computer Science
February 13, 2006
In Partial Fulfillment of the Requirements for the Degree of
Master of Engineering in Electrical Engineering and Computer Science
at the Massachusetts Institute of Technology

ABSTRACT

Molecular and synthetic biologists have increasing demand for large, high-fidelity constructs of synthetic DNA. Recent developments in harvesting oligonucleotides from DNA microarrays has proven that these can be assembled into large constructs of DNA for costs significantly less than traditional methods. This thesis presents a complete platform for the photoelectrochemical cleavage of protecting groups during in situ DNA synthesis. Photoelectrochemically patterned microarrays of phosphoramidite dye were produced with spot sizes of 100 um. This technology holds the potential for lowest cost per base pair of DNA produced over alternative microarray technologies, and may prove to be useful in de novo synthesis of large DNA constructs.

Thesis Supervisor: Joseph M. Jacobson
Title: Associate Professor, Department of Media Arts and Sciences
Acknowledgements

To Brian Chow I am truly grateful to you for sharing your extraordinary knowledge of organic chemistry and surface science, and your help in the final throws of troubleshooting various aspects of this project.

I would also like to express my sincere gratitude to David Kong for his generous donation of mask designs, Jae-Bum Joo for his help in SEM imaging, Kurt Broderick at the MTL for his help in device processing, Joseph Jacobson for allowing me to pursue this very cool project, and Lindsay Giorgi for her constant support throughout this project and help in proofreading this document.

This project would not have been nearly as complete without the generous donation of an automated DNA synthesizer from the MIT Biopolymers Lab.

I would also like to thank John and Sharron Emig because, well, not a day goes by that I forget to appreciate how they have helped me to succeed.
Table of Contents

1.0 Introduction.......................................................................................................... 5
1.1 Microarray Technology Concepts......................................................................... 6
   1.1.2 Traditional Oligonucleotide Synthesis: The Phosphoramidite Cycle ............ 7
   1.1.3 In Situ DNA Microarrays: Phosphoramidite Chemistry with a Twist ....... 8
1.2 Photoelectrochemical Arrays: A New DNA Microarray Synthesis Platform . 12
2.0 Photoelectrochemical Array Platform Development.............................................. 14
   2.1 Electrochemistry .......................................................................................... 15
      2.1.1 Development of Instrumentation and Fluidics ..................................... 15
      2.1.2 Materials selection ............................................................................ 15
      2.1.3 Three Electrode Fluidic ................................................................. 16
      2.1.5 Difficulties of Electrochemical Analysis ......................................... 19
      2.1.6 Selection of Reagents for Electrochemical Trityl Deprotection .......... 20
   2.2 Design and Fabrication of Semiconducting Substrates .................................... 22
      2.2.1 Photoconductive Processes and Properties ........................................ 22
      2.2.2 Stability During Electrochemical Processing .................................... 25
      2.2.3 High resolution .............................................................................. 26
      2.2.4 Facility of Production ..................................................................... 27
      2.2.5 Fabrication of Semiconducting Substrates ....................................... 27
   2.3 Photoelectrochemistry .................................................................................. 31
   2.4 Surface Chemistry for Oligonucleotide Linkage ............................................. 33
      2.4.1 Porous Attachment Layer ............................................................... 33
      2.4.2 Organosilane Coupling Agents ....................................................... 37
   2.5 Chemical Reagent Delivery ........................................................................... 41
   2.6 Maskless Lithography System ....................................................................... 42
   2.7 Supporting Software for Controlled Patterning and Synthesizer Integration. 45
3.0 Results ................................................................................................................ 47
   3.1 Photoelectrochemical Trityl Deprotection and Phosphoramidite Coupling ... 47
4.0 Discussion ............................................................................................................. 51
5.0 Conclusion .......................................................................................................... 54
6.0 References ........................................................................................................... 56
Appendix A................................................................................................................. 59
1.0 Introduction

Molecular and synthetic biologists are increasingly relying on the use of large constructs of synthetic DNA for use in research pursuits as diverse as vaccine development, protein expression, and genetic circuit fabrication [1-4]. De novo constructs as large as 31,656 base pairs have allowed biologists to express the extraordinarily large polyketide synthase gene cluster [5], and the entire 21 genes that encode the E. coli 30s ribosomal subunit [6]. Combined with the huge amount of genomic sequence data currently available, the inexpensive and accurate de-novo synthesis of large DNA constructs will allow synthetic biologists to have unprecedented control in the design of novel genetic circuits and the expression of genes from a variety of sources in model organisms. Nevertheless, significant hurdles still remain in the acquisition of inexpensive, high fidelity synthetic DNA. Although protein mediated error correction has shown impressive results in improving the fidelity of chemically synthesized DNA [7, 8], access to inexpensive oligonucleotides for gene synthesis is a problem for many researchers.

The creation of entirely synthetic constructs of DNA relies on the enzymatic assembly of multiple smaller oligonucleotide fragments of approximately 50 bases each [1]. Traditionally, construction oligonucleotides are synthesized on a column of porous glass beads using phosphoramidite chemistry [9]. At a current published cost of about 32 cents per base pair (Invitrogen, Carlsbad, CA), the synthesis of a large construct such as 100kb, costing around $64,000 in oligonucleotides alone, is not feasibly repetitive on an academic or industrial budget. Massively parallel in-situ oligonucleotide synthesis on DNA microarrays can produce oligonucleotides at a cost of less than 1 cent per base pair [10-13], but only recently has it been demonstrated that these oligonucleotides can be cleaved from the microarray chip and used to assemble genes [6, 14, 15]. Although the fidelity of these array synthesized nucleotides is rather low, it is expected that optimization of array synthesis parameters, combined with techniques developed for error correction, will provide the ability to produce extremely high length synthetic DNA constructs that were previously economically infeasible to synthesize.

Many methods exist for the production of DNA microarrays, although each technology has its advantages and drawbacks. Nevertheless, for many researchers, acquisition of custom designed arrays for gene synthesis is expensive and sometimes very difficult. This project was aimed at developing a new technology for DNA array synthesis to rapidly produce lowest cost, high-density DNA microarrays, to ultimately provide synthetic biologists with oligonucleotides for use
in genomic scale synthesis. In doing so, an entirely new array synthesis technology was invented, using a photoelectrochemically guided spatially defined reaction chemistry, along with several other techniques that may be useful for researchers in the biomaterial array field. Culminating in the verification of a spatially selective detritylation reaction, this thesis will demonstrate that this is a viable platform for the synthesis of inexpensive, high-density, custom DNA microarrays.

1.1 Microarray Technology Concepts

Fundamental to all DNA microarrays is the spatial direction of DNA placement or synthesis. The techniques for DNA localization fall under two broad categories - *in-situ* DNA synthesis, whereby the oligonucleotides are synthesized via modified phosphoramidite chemistry, and spotted array synthesis, whereby pre-synthesized or extracted DNA is affixed to a solid support via spotting or inkjet methods. *In-situ* arrays can synthesize a million different oligonucleotide sequences for less than the cost of a single plate of 96 different oligonucleotides synthesized with traditional methods – *in situ* methods simply produce the most addressable and unique chemistry per cost ever known. This is in contrast to spotted arrays, which require DNA to be extracted from organisms or synthesized from conventional methods, so there is no cost savings per nucleotide produced. Furthermore, many of the new *in-situ* synthesis methods are quite general in technique, leaving the field wide open to the synthesis of different types of biopolymer and combinatorial chemistry arrays. For these reasons this project is aimed at building an optimal *in-situ* array synthesis technology.

Several techniques for *in-situ* DNA array synthesis have been reported in the literature: photocleavable 5’ and 3’ protecting groups [11] [10]; deprotection of the acid labile trityl groups by photogenerated acids [12]; electrochemical acid generation via integrated circuitry [16], [17], ink-jetting reagents [18], and micro contact printing [19]. Every one of these techniques utilizes decades old chemistry with a twist – a spatially confined reaction with resolutions on the order of 10-100μm.
1.1.2 Traditional Oligonucleotide Synthesis: The Phosphoramidite Cycle

All \textit{in-situ} techniques follow derivatives of the phosphoramidite synthesis cycle invented by Beaucage and Caruthers (1981) and perfected over the past 25 years [9, 20]. The traditional phosphoramidite synthesis cycle (see Figure 1.1) allows for single-reaction combinatorial synthesis of DNA oligonucleotides. The reaction is based on techniques for solid-phase chemical synthesis, in which molecules are covalently or ionically bonded to a stable glass or polymeric support structure during washes with appropriate reagents. During each phosphoramidite cycle, a specific deoxyribonucleoside phosphoramidite is activated with a weak acid, tetrazole, to react rapidly with a free hydroxyl on the surface of the support in the first cycle, and then the growing DNA strand in subsequent cycles. Since at each step any of the four DNA nucleoside phosphoramidites can be added, any arbitrary strand of DNA can be produced. Improper couplings yield free hydroxyls which are capped with a solution of acetic anhydride and methyl imidazole so that failure sequences cease to grow and can be easily purified from success sequences. The coupled phosphoramidites form a phosphite triester bond, which is oxidized to a more stable phosphate triester with a solution of iodine, pyridine and water. Each phosphoramidite has a reactive nucleophile for the subsequent coupling that is traditionally capped with dimethoxytrityl, so that repeated couplings are avoided. However, in order to react with the next phosphoramidite, the dimethoxytrityl is removed with a relatively strong acid solution of dichloroacetic acid. Upon de-tritylation, the growing strand yields a nucleophilic hydroxyl ready to react with the next phosphoramidite. Since the nitrogenous bases of nucleosides have nucleophiles as well, the corresponding phosphoramidite forms employ a variety of base cleavable protecting groups that prevent unwanted side reactions. After repeating the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure_1.1}
\caption{The phosphoramidite synthesis cycle.}
\end{figure}
aforementioned cycle and removing the base cleavable protecting groups in concentrated ammonium hydroxide at the very end of the synthesis, oligonucleotides upwards of several hundred nucleotides long have been produced [21].

Although synthesizing long sequences with the phosphoramidite cycle is in theory easy to accomplish, there are many failure mechanisms that decrease the overall yield of DNA. At any step, an incomplete reaction yields a failed product. Furthermore, the phosphoramidites and capping reagents are extremely reactive with water, so the entire system must be kept under an inert atmosphere. Any water in these reactions has disastrous effects on the yield of the synthesis. Generally automated synthesizers, such as the ABI 394 are used to perform these synthesis cycles under appropriate conditions. However, even the best synthesizers have failure rates of about 1/100 for each entire cycle. Therefore, after a very long synthesis such as 100 cycles, the expected amount of perfect DNA is approximately $0.99^{100}=36.6\%$ of the initially derivatized support. If the coupling efficiency falls to 96%, only 1.6% of the initially derivatized support has perfect DNA sequences. In practice, phosphoramidite synthesis requires both a significant amount of optimization to ensure good yields at the end of a synthesis cycle, and a mechanism for purifying perfect sequences from failures.

1.1.3 In Situ DNA Microarrays: Phosphoramidite Chemistry with a Twist

One key to the phosphoramidite cycle is the deprotection of the newly added phosphoramidite nucleotide. Each newly added phosphoramidite includes a stable protective group (traditionally dimethoxytrityl) at its reactive 5’ end so as to prevent repetitive additions of the same phosphoramidite. After the chamber is washed, a mechanism appropriate to the protective chemistry is employed to remove this protecting group. Traditionally, dichloroacetic acid is used to remove the acid cleavable dimethoxytrityl protecting group. The deprotection scheme exposes the reactive hydroxyl group, allowing for the addition of another phosphoramidite.

Foder et. al (1991) were the first to recognize that if the oligonucleotides were confined to a 2 dimensional solid support, such as glass or silicon, then traditional mask photolithography techniques and newly-developed photo-cleavable protecting groups could be utilized to selectively determine which oligonucleotides are exposed to the acid deprotection step. Effectively, this allows for the selective addition of a specific phosphoramidite to those locations,
as in Figure 1.2. Furthermore, since lithography techniques allow for extremely high resolution spatial selectivity, hundreds of thousands of different oligonucleotides could be produced in a square centimeter, allowing for a cost effective manner of producing many different addressable oligonucleotides. Thus the first two dimensional, spatially directed, in-situ synthesized DNA microarrays were invented. This technology is marketed by Affymetrix (Santa Clara, CA).

Since the inception of the first microarray, several other technologies have been invented, all oriented toward developing different mechanisms for spatially defining reactions at each of the thousands of addresses on an array. Each of these synthesis technologies have their own advantages and drawbacks, with the metrics mainly defined by the synthesis yield (quality of oligonucleotides), the resolution (spots/square centimeter), and the costs of production. Moreover, expanding interest in custom microarrays for both hybridization and gene synthesis have spawned a rapidly growing market. Customizable arrays are difficult to manufacture with the original Affymetrix process because each base addition requires four separate lithographic masks, corresponding to the four possible nucleotide additions. For an array of 50-mer oligonucleotides, this corresponds to 200 masks and exposure steps. Therefore, the creation of an entirely new chip is a time consuming and expensive process with the original technology. Hence, Affymetrix has focused on markets that have enormous demand for standard arrays, such as personalized genomics and research on common organisms. The use of masks allows for the highest resolution available for these standardized arrays. However, for those that wish to make an entirely new chip for use in hybridization to the genome of a newly discovered organism, or for use in gene or genome synthesis, the technology does not scale well.

Figure 1.2. Schematic of a spatially selective deprotection.
With the protecting group removed on the "A" section, the reactive hydroxyl will couple with an incoming phosphoramidite. Since the other sites are protected with DMT, no coupling occurs at those locations.
Singh-Gasson et al (1999) pioneered the use of the digital micromirror (DMD) array from the newly developed Texas Instruments DLP technology to allow for a mask-less approach to the original UV-photocleavable system. In this embodiment, a computer can control a digital projector with a 1024 x 768 array of mirrors with a pixel size of 16 um by 16 um, allowing for almost 1 million configurable spots per square centimeter, in effect acting as a virtual mask. This technology is now marketed by NimbleGen Inc. (Madison, WI).

Although the UV photocleaveable system is very elegantly designed, especially with a mask-less approach, the UV deprotection efficiency does not compare favorably with the traditional dimethoxytrityl deprotection, and the photocleavable phosphoramidite reagents are extremely costly to produce - upwards of $400 for the synthesis of a single chip [22]. Furthermore the exposure times are very long, on the order of 10 minutes, and repeated exposures of the oligonucleotides to ultraviolet light may cause structural damage to the nascent strands.

Several others have demonstrated customizable array technologies as well. One technique pioneered by Gao, et. al, [12] utilizes a photo-generated acid (PGA) and a digital micro-mirror device (DMD). In this embodiment, incident light from a DMD initiates acid generation in solution at the selected sites. Since the photo-initiated acid is generated volumetrically and not only on the surface of the chip, diffusion of the acid becomes a limiting factor in determining density. As a result, these chips have yet to demonstrate the same spot density as their photocleavable counterparts because each chip requires complex fluidics to spatially confine the generated acid [23, 24].

Other methods for *in situ* synthesis exist such as micro-contact lithography [19] and ink-jetted reagents [13]. Micro-contact printing of phosphoramidites allows for extremely high-resolution chip production, but this technique is limited in its versatility because it requires the creation of a new stamp for each base added. To date, arrays generated by ink-jetted reagents have demonstrate high sequence fidelity, but are limited in spot density by the smallest droplet size generated by piezo print heads and placed reliably by a mechanical system. The inkjet process also takes a very long time to produce a single chip.

Dill et. al, (2004) were the first to demonstrate a complete integrated circuit array of electrodes to guide an electrochemical oxidation at each array spot in order to synthesize an oligonucleotides array (this technology is now marketed by Combimatrix, Mukilteo, WA). The oxidation reaction
releases free protons, generating a local acid gradient at the surface of the chip, and allowing for the acid catalyzed deprotection of dimethoxytrityl. Since this system parallels the traditional method of phosphoramidite synthesis, expensive UV photocleavable reagents are avoided, and the reagent cost is significantly lower in cost than in the Affymetrix/Nimblegen systems. Furthermore, electrochemically generated acid deprotects trityl groups on the order of minutes as opposed to tens of minutes for the photocleavable systems. Nevertheless, this system greatly increases device complexity because each chip is a completely addressable integrated circuit array that must be fabricated with traditional fabrication techniques. Although mask-less, the technology has all array addressing on chip. Therefore reagent costs are in effect shuttled to the substrate costs – chips enter the market around $1000 each.

Egeland and Southern (2005) utilize a similar technique in which the electrically generated acids are generated on a print-head array of iridium electrodes close to a glass substrate that has the nascent oligonucleotides. Thus the acid is generated at the print-head and diffuses toward the substrates to selectively deprotect dimethoxytrityl. This system has demonstrated a resolution comparable to the aforementioned electrochemical technique, and the device should be reusable, yielding a minimal substrate cost. However, the technology has yet to demonstrate a large scale two-dimensional array synthesis.
1.2 Photoelectrochemical Arrays: A New DNA Microarray Synthesis Platform

I have developed a virtual electrode system, similar to the electrochemical technique of Dill et. al and Egeland and Southern, but utilizing a photoconductive substrate that spatially controls electrochemistry via illumination with light. Photoconductive patterning has previously been used to pattern and move biomolecules and polymer beads [25, 26], move fluids in microfluidics by opto-electrowetting [27], and control electrochemistry for potentiometric analyte detection [28-30], but has yet to be utilized to spatially-control electrochemically generated reagents during solid phase synthesis.

![Diagram](image)

**Figure 1.3** An example platform for photoelectrochemical array synthesis. Patterned light incident on a photoconductor creates charge carriers and subsequently a localized surface potential sufficient to electrochemically generate acid, which then locally removes dimethoxytrityl protecting groups.

In such a system, substrate costs are minimized – simple thin film technologies can be used, lending substrates to be fabricated for a miniscule price circa $30 each. Additionally, since it takes advantage of the traditional, inexpensive phosphoramidite reagents, the reagent cost is also considerably cheaper than that of those systems requiring photo cleavable protecting groups. The challenges of building such a technology are significant, as it lies at the intersection of several fields (Figure 1.3). The development of this new technology of microarray synthesis has the
potential to allow for the most inexpensive creation of high density microarrays in a maskless manner, ultimately to be used later in gene and genome synthesis.

**Photoelectrochemical Array Synthesis**

![Diagram of intersecting fields: Semiconductor Physics and Device Fabrication, Electrochemistry, Solid Phase Chemical Synthesis, Optical Engineering, with Photoelectrochemical Array Synthesis at the intersection.]

**Figure 1.4** Photoelectrochemical array synthesis lies at the intersection of several fields.
2.0 Photoelectrochemical Array Platform Development

Several engineering and chemical process related pitfalls were overcome to make this technology a reality (Table 2.1). This project required semiconducting substrates capable of withstanding the rigors of phosphoramidite synthesis (repeated exposures to acids and bases), an electrochemical readout and control system, the evaluation of electrochemical oxidation/reduction couples and electrolytes, the construction of optics and mechanics for aligning and focusing light from a digital micromirror device, fluidics for reagent delivery, the synthesis of a porous matrix for the attachment of DNA, development of assays for surface functionalization, and the design of software for directing the synthesis cycles, projecting light, and controlling the electrochemistry (See Figure 2.1).

![Diagram of photoelectrochemical deprotection scheme]

**Figure 2.1** Overview of photoelectrochemical deprotection scheme.

<table>
<thead>
<tr>
<th>Device Design and Fabrication</th>
<th>Solid Phase Chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials Selection</td>
<td>Porous Attachment Surface</td>
</tr>
<tr>
<td>Adequate Photoconductive Response</td>
<td>Surface Chemistry</td>
</tr>
<tr>
<td>Surface Stabilization</td>
<td>Reagent Delivery</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Optics and Mechanics</th>
<th>Electrochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 Projection Optics</td>
<td>Redox Chemistry</td>
</tr>
<tr>
<td>Alignment Optics</td>
<td>- Non-aqueous acid/base chemistry</td>
</tr>
<tr>
<td>Software Control</td>
<td>Instrumentation</td>
</tr>
<tr>
<td></td>
<td>- Potentiostat Construction</td>
</tr>
<tr>
<td></td>
<td>- CV Measurement Hardware</td>
</tr>
<tr>
<td></td>
<td>- Three electrode fluidic</td>
</tr>
</tbody>
</table>

**Table 2.1** Major hurdles of photoelectrochemical array synthesis.
2.1 Electrochemistry

The electrochemical aspect of this project necessitated research and development into the appropriate selection of reagents, materials for device and assembly fabrication, and also creation of tools in both hardware and software to measure and analyze electrochemical currents. Proper instrumentation is crucial for electrochemical analysis, therefore a three electrode electrochemical cell and fluidic suitable for phosphoramidite synthesis, and a potentiostat circuit were built. After verification that instrumentation was effective, an electrochemical redox system was chosen and characterized to find proper working conditions for the deprotection of trityl groups.

2.1.1 Development of Instrumentation and Fluidics

Electrochemical methods require the precise control and measurement of voltage and current to an electrochemical cell in order to provide analytic feedback of the chemical reactions taking place in the cell. This control is typically accomplished through the use of a three electrode electrochemical cell, and a potentiostat circuit for precisely controlling the potential and measuring the current in the cell. A potentiostat functions to maintain a constant voltage between two electrodes while driving current through a different electrode. Typically, the reference electrode is non-polarizable, and no current flows through it, so it can be used to measure a somewhat absolute potential in reference to the working electrode. This allows for precise measurement of working electrode voltages and currents in spite of side reactions that may happen at the counter electrode (such as oxidation of the metal). In this project, the photoelectric active device is the working electrode. Since the potentiostat was always used during electrochemical deprotection in order to monitor working electrode (semiconducting device) current, it was necessary to build a three electrode cell that was capable of sustaining phosphoramidite chemistry.

2.1.2 Materials selection

Since oxidation and reduction reactions require the motion of electrons to and from electrode materials, it is important to select materials that can act as perfect inert electrodes during these processes. The materials used in most electrochemical studies are platinum, palladium, iridium and gold, as they are extraordinarily resistant to electrochemical modification. Due to the high
prices of these materials, other less noble metals can also be used, such as nickel. Originally it was thought that nickel alloy stainless steel would be resistant enough to electrochemical oxidation that it could suffice as a counter electrode, but experiments proved that this material rusted rapidly under the conditions evoked during electrochemistry. Therefore a pure nickel counter electrode was used for the remainder of the experiments. It is particularly important that the working electrode is perfectly inert, as it is used to measure electrochemical currents. In my initial tests of the nickel electrochemical cell and potentiostat circuit a pure platinum foil was used as the working electrode. Subsequent tests utilized the semiconductor device described in section 2.2.

2.1.3 Three Electrode Fluidic

A custom machined fluidic was designed to hold substrates while meeting the stringent requirements of solvent resistance during phosphoramidite synthesis, supporting a three electrode setup for electrochemical deprotection and measurements, and including an optical window for substrates illumination by a light source. Since the phosphoramidite synthesis cycle exposes the fluid handling components to oxidants, acids, bases and solvents such as dichloromethane and acetonitrile, it is necessary that all parts exposed to these solutions are composed of inert materials such as glass, Teflon (or other fluorocarbon polymers), platinum, gold, and type 316 stainless steel. Additionally, the fluidic required an optical window to the semiconductor device surface, through which the semiconductor is illuminated, and proper electrical contacts to the device and the solution. A variety of fluidics were designed to support the synthesis. There were two main fluidic designs proposed, although in the end only one was used for the results published. One design considered was front side illumination in which light strikes the substrate/solution interface. The alternative was positioning the light source such that illumination occurred from the opposite side of the substrate, in this case the side exposed to air. Each design requires a complimentary photoconductive substrate design, since the illumination source must be capable of generating a strong enough response in the photoconductive layer without being blocked by excessive absorbing materials.
The final back side illumination fluidic comprised a rough nickel surface with connections to standard threaded HPLC equipment and a Kalrez o-ring (DuPont, Wilmington, DE) that served as the seal between the semiconducting device and the nickel fluidic (Figure 2.3). The fluidic operated in two modes: the testing mode and the synthesis mode. In the synthesis mode, the fluidic was secured to a vacuum alignment chuck that held the fluidic. In testing mode, an acrylic washer was used to compress the device or a platinum foil to the fluidic. The non-electrochemically active section of the nickel was covered with a Teflon sheet glued with Viton adhesive caulk. A hole was drilled in the backside of the nickel fluidic for the insertion of the reference electrode. The nickel base of the fluidic also acted as a counter electrode. In this manner, the ITO/semiconductor device serves as both the optical window and the back side of the fluidic, while its bus-bar is exposed to the outside of the fluidic for an electrical contact.

2.1.4 Potentiostat Circuit

A simple active I/E converter potentiostat circuit was developed that is based on the design in [31]. There were a few minor alterations to the circuit. The supply lines to the OP27’s were connected with 0.2 μF capacitors. An 80 Ohm resistor was placed in series to the power source at the V2- and V1- terminals of the current buffers in the control amp in order to limit the maximum current to 140 mA and prevent accidental current overload. The control voltage of the circuit was connected to a source/measurement unit on HP 4156A (Hewlett Packard, Palo Alto, CA).
A simple Labview program was written to access the instrument through GBIP and save the waveform.

Figure 2.4 Circuit model for potentiostat circuit. $V_{\text{sense}}$ and $V_{\text{in}}$ were either connected to a HP 4156 Semiconductor Analyzer for analytic purposes or the NI 6033E during deprotection bias control.

The output voltage ($V_{\text{sense}}$) was measured and recorded with a voltage measurement unit on the HP 4156A across the sense resistor in the I/E converter. Thus the output voltage is representative of the current passing through the working electrode. The sense resistor was varied according to the sensing range desired ($V_{\text{source}}/R = \text{max current in amps}$). The measurement resolution was approximately equal to $5 \times 10^{-4}/R$ in amps, and was limited only by the noise in the electrochemical apparatus and potentiostat circuit (the HP 4156A was capable of 100 μV resolution). A 1k sense resistor was used for the majority of the experiments, yielding a dynamic
range from 500 nA to 15 mA, and current resolution of 500 nA, which compares with commercial
potentiostats and cyclic voltammetry equipment. Larger sense resistors (10kΩ, 100kΩ) were
used when higher resolution was necessary with the concomitant drops in dynamic range.

The potentiostat circuit was tested with the electrochemical cell previously described, with a
Ag/AgCl reference electrode and a platinum working electrode inserted in their appropriate
positions. The cyclic voltammogram of 2mM ferrocene with 50 mM tetrabutylammonium
hexafluorophosphate in acetonitrile is shown in figure 2.5. Similarly a CyV of a 25 mM
hydroquinone/benzoquinone solution in the same salt/acetonitrile solution is seen in Figure 2.6.
Both of these readings clearly show the redox potentials comparable to those in the literature.
These cyclic voltammograms demonstrated that the potentiostat was functional in the regime
desired and would be adequate for the remainder of experimentation.

Figure 2.5 Cyclic voltammogram of
ferrocene on platinum.

Figure 2.6 Cyclic voltammogram of
hydroquinone/benzoquinone on platinum.

2.1.5 Difficulties of Electrochemical Analysis
The phenomenological aspects of applying a potential to an electrode in solution are difficult to
model. Applied potentials induce multiple effects simultaneously – electrolysis, ohmic
conduction, electrophoresis, double layer capacitance, and numerous other phenomena. Although
simple models exist to help describe current transport in electrochemical systems, such as in
Figure 2.7, these are mainly for analysis in impedance spectroscopy and they are not always
helpful for organic electrochemists. Electrochemical effects on arbitrary molecules, especially in
non-aqueous solutions, are largely uncharacterized. Even the mechanisms of acid reactions in a
non-aqueous solution are not universally understood. Therefore, it is wise to start with a system
that is already well characterized, such as the redox chemistry of ferrocene, or the redox mixture investigated by Egeland and Southern for electrochemical deprotection [17].

2.1.6 Selection of Reagents for Electrochemical Trityl Deprotection

In order to remove the dimethoxytrityl protecting groups, electrochemical generation of acid is required. The electrochemical oxidation of an amine or hydroxyl is yields free protons (Figure 2.8). Preferably, both the reduced and oxidized forms of this molecule are non-reactive with the growing DNA. A family of compounds that show remarkable stability, and electrochemical quasi-reversibility, are the quinones. These compounds have a gradient of redox potentials based on the number of aromatic rings and exposed hydroxyls (See Figure 2.9). Although many electrochemical techniques focus on the oxidation of hydroquinone, it is possible that these other quinones could be used for similar purposes under the appropriate conditions.

In selecting appropriate redox chemistry, the constraints of phosphoramidite synthesis should also be considered. Phosphoramidite synthesis is extremely sensitive to water content during the reaction, so the use of water or nucleophilic compounds in synthesis steps should be minimized. Furthermore, hydroquinone will slowly oxidize to benzoquinone in the presence of any oxidizing agents. Hence organic electrochemistry is preferred. Egeland and Southern demonstrated good
results with hydroquinone and benzoquinone as the redox couple in a solution of acetonitrile with tetrabutylammonium hexafluorophosphate as the supporting electrolyte [17]. Oxidation of hydroquinone yields benzoquinone and two free protons (Figure 2.8), while reduction of benzoquinone to hydroquinone yields a basic dianion. The organic salts serve to enable better conduction through the organic solvent, stabilize the potential at the electrode surface, and minimize the Debye length. The mixture used for all experiments, unless noted, consisted of 12.5 mM hydroquinone and 50 mM Bu₄N⁺PF₆⁻.

Acid generated at any surface is expected to rapidly diffuse into the bulk solution. With an end goal of spatial reaction localization, this is a problem that can be addressed through diffusive barriers or selection of appropriate buffering chemistries. Depending on the amount of diffusion experimentally observed, these adjustments to the system may need to be made in tandem or individually. Potential diffusion barriers could be gel delivery of reagents, or structures microfabricated in glass or silicon or other materials, such as wells at the sites of electrochemical acid generation. Chemical solutions to the acid diffusion problem require buffers or acid scavengers. Organic bases such as triethylamine or pyridine could function as appropriate scavenging materials if necessary. As will be discussed in later chapters I utilized 1 mM triethylamine as a scavenger in some experiments.

![Diagram of various members in the quinone family and associated oxidation potentials.](image)

**Figure 2.9** Various members in the quinone family and associated oxidation potentials.
2.2 Design and Fabrication of Semiconducting Substrates

The goal of this project was to develop an entirely light addressable electrochemical synthesis platform, requiring that at least one part of the device necessitated photo-switchable electrochemistry. Therefore the design of a suitable semiconducting substrate with adequate contrast ratio between light and dark electrochemical currents was designed and fabricated.

As a result of the inherent complexity of a novel photoelectrochemical system, it is necessary to decompose the system into simpler components. The four requirements for the semiconducting device were:

1. Adequate contrast ratio and photocurrent generation under potential ranges, light power and spectrum available.
2. High resolution of the photo-selective response.
3. Stability during DNA synthesis and electrochemical reaction.
4. Facility of processing.

2.2.1 Photoconductive Processes and Properties

The novel aspect of this system is the light addressability, thus considerable time was spent on choosing an adequate semiconducting material. Without sufficient contrast ratio in electrochemistry between light and dark spots, photoelectrochemical arrays are not possible. Crystalline and amorphous solids were investigated, as well as thick and thin film semiconductors. Furthermore various heterojunctions were postulated, but ultimately a rather simple amorphous silicon substrate with a patterned platinum protective layer was used, meeting the original goal of simple, low cost substrates.

Photoconduction or photocurrent occurs in a semiconductor when an incident photon has sufficient energy to move valence electrons to the conduction band. This is known as the band-gap of the semiconductor:

\[ E_{\text{gap}} = \frac{hc}{\lambda} = \frac{1.24}{\lambda(\mu m)} \]

Charge carrier generation is dependent upon the amount of photons absorbed that exceed the band gap of the semiconductor. Since the absorption depth of a photon is a function of wavelength, it
is desired that the semiconductor layer be of sufficient depth to absorb enough photons to generate a high enough current through the photoconductor at a given bias potential to react at the electrolyte interface. Furthermore, since high contrast ratio in current is desired, a photoconductor with sufficiently high on/off current at room temperature is necessary, which requires both that thermal generation of free charge carriers is minimized and optical absorption is very high. Although the light source is customizable, it was preferable to have a light source that was both easily controlled and matches the optimal absorption peak in the semiconductor. Since a stock digital projector outputs a colorful spectrum between the infrared and the ultraviolet (as seen in Figure 2.10(a)), semiconductors with band gaps of sufficiently low energy to absorb most of the light in this region are preferable and correspond to band gaps between 1.8 eV and 3.26 eV. However, if the photoconductor has a band gap corresponding to energies of photons in the near ultraviolet, it is possible to swap the stock bulb of a projector to one with more UV output, such as the HBO 103/45. Output spectrum from this modification is seen in Figure 2.10(b).

![Figure 2.10(a) Output spectra of Infocus X1 projector after condensing lens, UV filter and color wheel removed.](image-url)
Figure 2.10(b) Output spectra of Infocus X1 projector after condensing lens, UV filter and color wheel removed and the stock halogen bulb replaced with an HBO 103/45. Notice the increase of near UV emission with the mercury bulb.

In this project, a specific reaction is desired that can produce free protons to acid-cleave chemical protecting groups. The electrochemical reaction occurs at a potential necessary to transfer electrons from the organic molecule to the substrate surface. This potential may be applied by an externally exposed voltage source or from an internal electric field, such as in photovoltaic device. If the device has an intrinsic electric field such as a PN junction, a Schottky junction, or an electrode-electrolyte interface, the electric field can separate the charge carriers enough to flow through an external circuit. If the electric field is high enough, these electrons may have enough power to react at the electrolyte interface. Thereby charge carriers generated via the photoelectric effect are shuttled to react at the electrolyte interface by this built-in electric field.

Seemingly, contrast ratios should be better under photoconductive gain regimes, and when a photoelectric switch is used to control a more non-linear effect. In a simple photoconductor, the application of a potential across a semiconductor can result in extreme photoconductive gain depending on the potential and device geometry. When combined with an electrochemical system, the semiconductor operates in a linear gain regime, gated by the non-linearity introduced by the requirement of an adequate surface potential for electrochemical oxidation. Below the oxidation potential of a redox couple in solution, almost no oxidation takes place. Operation at the oxidation potential yields a current density dependent on the relative concentrations of the reduced and oxidized forms in solution, while above the oxidation potential of the molecule yields an exponential increase in current densities. This type of system produces optimum contrast ratios, since light cannot be easily modulated to 100% contrast ratios, but need not be,
because the electrochemistry is gated by the redox potential of the molecules of interest. This system allows a linear light switch to control extremely non-linear electrochemical effects – the semiconductor acts as a controllable voltage source, while the electrochemistry has a non-linear current-voltage relationship. Therefore, a small increase in conductivity of the photoconductor can control a very large current by slightly changing the potential at the surface of the substrate.

2.2.2 Stability During Electrochemical Processing

Photoelectrochemical catalysis involves harsh processing conditions and devices must serve as stable electrodes in an electrochemical reaction. Thus, the surface of the device must be electrochemically inert while current is passed through it, otherwise the surface will degrade unpredictably, and electrochemistry will be difficult to measure or repeat reliably. Furthermore, semiconductors are susceptible to similar effects through photo-degradation. Of the many semiconducting materials available, anatase titanium dioxide fits these many requirements ($E_{\text{gap}} = 3.2 \text{ eV}$). As a result of its extreme stability, anatase titania has been vigorously pursued as a photoelectrochemical solar cell [32], though these cells require dye-based sensitizers for visible spectrum light absorption. It has been reported that transfer reactions with dye-sensitized semiconducting titania without externally applied potentials yields poor contrasts ratios for photoelectrochemical catalysis from visible wavelength photons [33]. Therefore it was concluded that a photoelectrochemical synthesis would require a photoconductive gain through the imposition of an externally applied bias.

Unfortunately, materials with high absorbance in the visible are almost universally poor during electrochemical reactions, as applying bias to a semiconductor surface can cause bond rearrangement and oxidation, and exposure to light can cause photo-degradation [34]. Hence, until materials permit, the optimal device is composed of multiple materials, such that the photoconductive layer and a more stable electrochemical layer can be separated. An amorphous silicon photoconductive layer with a protective covering of titanium dioxide or platinum is an attractive design because of the relative ease and cost of device processing. However, pitfalls of these designs are apparent – a conducting metal as a protective surface requires patterning into microelectrodes so that the active surface is localized, whereas semiconducting titania requires enough light of the appropriate band gap incident on to the titania, so that the protective covering is sufficiently conductive.
2.2.3 High resolution

The resolution of the process is defined by diffusion of charge carriers in the photoconductive layer, and diffusion of electrochemically generated acid. Neither of these processes prevent achieving resolutions on the scale of the best currently available DNA microarrays, as current electrochemical arrays can achieve spot sizes of 45 um, while the ambipolar diffusion length in the photoconductive layer can be as low as 115 nm, which corresponds to a much larger spot size in practical photoconduction [17, 29, 35]. It has been reported that thin film technologies theoretically yield the highest resolution for light addressable potentiometric sensors [29]. It is hypothesized that this is a result of charge carrier diffusion in the bulk of the semiconducting material – the thicker the substrate, the more likely charges will diffuse laterally before transferring to solution. Since LAP sensors are remarkably similar in concept to a photoelectrochemical synthesis array, these calculations are directly applicable to the goals of this project. Theoretical calculations and experimental results yield a minimal spot size of 15um [29]. Thus, photoelectrochemical synthesis technology is not limited by semiconductor physics in scaling down to the resolution of current commercial DNA arrays.

2.2.4 Facility of Production

Thin film technologies offer the lowest cost and in general the easiest handling of all semiconducting materials. Thin films of amorphous solids can be attained through sol gel chemistry [32] such as in the Graetzel cell, or through various vacuum deposition technologies, such as amorphous silicon solar cells. Thin film technologies also allow flexible or textured base materials if that is a design requirement.

Multiple patterning steps should be avoided as they contribute to the cost of production significantly. This is particularly apparent when scaling from a single mask step to multiple mask steps as mask alignments are then necessary for production.

The optimal approach to photoelectrochemical arrays includes thin film technologies, limited lithography steps, semiconductors with band gaps between 2.0 and 3.2 eV, and inert conductive materials at the electrolyte interface. Of the potential materials available, amorphous silicon satisfies these the facility of production requirements above. Amorphous silicon is a very well characterized photoconductor and deposition technologies are readily accessible commercially.
and academically. Furthermore, the oxidation of silicon yields a highly inert silicon dioxide surface [36], which was later determined to be electrochemically inert under moderate field strengths as well. Inert metals such as platinum can be easily patterned onto the silicon with photoresist lift-off techniques. With a single patterning step, these substrates should be extremely inexpensive to produce. To this end, I fabricated an amorphous silicon photoconductor array for further experimentation.

2.2.5 Fabrication of Semiconducting Substrates

Two illumination procedures were considered, one with solution side illumination and one with substrate side illumination. Since two illumination procedures were considered, multiple designs were proposed for the substrate – some utilizing semiconductor on steel, or on transparent conducting oxide, some using titanium dioxide as a protective surface, or some using platinum as a protective surface. Ultimately, amorphous silicon was chosen as the semiconductor because of the aforementioned reasons: its unusually high optical absorption in the visible region, the ease of access to processing tools and materials, and the high photoelectrochemical resolution possible with thin film technologies. Additionally, due to the ease in handling the back-side illumination fluidic and substrate, the platinum protected, back-side illuminated device was used for all experiments.

The back-side illumination device requires an optically clear, conductive window for incident light to generate sufficient charge carriers in the silicon. I chose indium tin oxide on glass slides as the main support for the substrates as it is easily obtained commercially and inexpensive. The substrates were further processed by thin film deposition of amorphous silicon, patterning with photoresist, and then thin film deposition and subsequent lift-off of platinum, as seen in Figure 2.11.
It was found that strict cleaning procedures were necessary to ensure adequate adhesion of the amorphous silicon to the ITO surface. ITO coated float glass slides (resistivity=15 ohms/cm²) (Delta Technologies, Stillwater MN) were cleaned by ultrasonication for 15 minutes in a 20% v/v solution of ethanolamine and water to remove residual organics and particulates. Slides were then rinsed clean four times in water, then rinsed once with isopropyl alcohol, blown dry with a stream of nitrogen, and then stored in a dry container.

Amorphous silicon was deposited by plasma enhanced chemical vapor deposition at the MIT Exploratory Materials Laboratory with a PlasmaTherm 700 Series PECVD (Unaxis Corporation, St. Petersburg FL). See table 2.2 for a detailed list of processing steps. The chamber was first cleaned with CF4 for one hour and cleared of fluorocarbons with a ten minute 200W nitrogen plasma to ensure a perfectly pristine chamber. It was then passivated with a precursory run of 20 minutes with silane plasma. The substrates were introduced into the device and heated to 250°C under vacuum. Prior to deposition, the substrates were cleaned in an oxygen plasma for three minutes, as it was reported in [37-39] that oxygen plasma treatment of ITO substrates may improve device characteristics by removing particulates and adsorbed organics, and improving hole injection by modifying the work function of the material. The substrates were then exposed
to the silane plasma for 240 minutes as indicated in Table 2.2. They were allowed to slowly cool in vacuum overnight. The films were measured to be between 0.9 um and 1.2 um with a Dektak 3 profilometer (Veeco Metrology Group, Chadds Ford, PA).

<table>
<thead>
<tr>
<th>Time</th>
<th>Vacuum</th>
<th>RF Power</th>
<th>5% CF₄ 95% O₂</th>
<th>5% SiH₄ 95% He</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>60</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>N₂ Purge (Defluorination)</td>
<td>10</td>
<td>300</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chamber Pre-Deposition</td>
<td>20</td>
<td>900</td>
<td>30</td>
<td>0</td>
<td>250</td>
</tr>
</tbody>
</table>

Substrate Insertion
| O₂ Clean | 3 | 300 | 0 | 0 | 25 |
| Si Deposition | 40 | 700 | 100 | 25 | 0 |
| Si Deposition | 100 | 900 | 30 | 0 | 250 |
| Si Deposition | 40 | 700 | 30 | 0 | 250 |

Anneal 1-2 Hrs. at 250 C
| Cool to 25C | 8 hrs | 700 |

**Table 2.2** Processing conditions for PECVD of amorphous silicon. Gas rates in sccm.

Failure to complete any of these steps resulted in significant delamination on the silicon, sometimes even before chemical processing steps. In other cases, the substrates would delaminate after exposure to bases, acids, water or organic solvents. For example, without ethanolamine pre-treatment, almost all of the substrates experienced delamination in air, and would delaminate within a few hours in water. Without a precursory run of the silane chemistry in the chamber, substrates would delaminate during the ethanol based silanization protocol, presumably because of some fluorocarbon adsorption onto the ITO during deposition of silicon. Without proper cooling (at least 4 hours), the substrates would crack and delaminate upon exposure to air. Needless to say, extreme precautions were taken at every step in the processing to ensure good adhesion. In the end, the silicon covered substrates were capable of soaking in an aqueous solution of trifluoroacetic acid for 72 hours, soaking in NoChromix (Godax, Cabin John, MD) in concentrated hydrosulfuric acid for 2 hours, sonication in acetone or ethanol, exposure to triethylamine, and exposure to 30 minutes of continuous cyclic voltammograms, during exposure of 150 mW/cm² lamp light, in 12.5 mM hydroquinone and 50 mM tetrabutylammonium hexafluorophosphate in acetonitrile from -1V to 1.7V vs. platinum reference electrode. In fact, these cyclic voltammograms clearly show limiting oxide formation also demonstrating the electrochemical non-conductance and inertness needed for the exposed silicon surfaces.
After PECVD deposition, the substrates were coated with AZ 4620 photoresist at a spin speed of 2000 rpm for 30 seconds after a ramp up of 10s. The slides were prebaked at 85°C for 60 minutes. The substrates were covered with a chrome mask with a pattern of a grid of 100 um squares on a 70 um pitch and exposed with a Zeiss mask aligner three times for 25 seconds with a 20 second cooling interlude between steps. They were developed in AZ 440 developer for 3-4 minutes, rinsed in deionized water and dried with nitrogen.

Directly after development of the photoresist, the substrates were placed in a Sloan PAK-8 electron beam evaporator and put under vacuum to 3.0x10^-6 T. After high vacuum was obtained, 15nm of titanium was evaporated onto the substrates as an adhesion layer, at 1 angstrom per second, and then 100 nm of platinum was evaporated onto the substrates at the same rate. The substrates were allowed to cool for thirty minutes in vacuum, and then thirty minutes under nitrogen before removal. After removal, the substrates were soaked in alternating solutions acetone and 1-methyl pyrrolidone for two days to lift-off the photoresist and extraneous platinum. The high processing temperatures during electron beam deposition highly crosslinked the resist, and it was necessary to sonicate the substrates in for 4 hours on the second day of soaking before the substrates were free of the resist and extraneous platinum. Due to the harshness of this processing step, it is acknowledged that better high-temperature resists such as OCG would be more suitable for this processing step. The substrates were then plasma cleaned (PlasmaSeries, Anatech LTD) at 100 mT for 10 minutes at 50 sccm O2 and 50W RF to remove any remaining organics. The resulting substrates (Figure 2.12) have two grids of 6,000 100x100 micron platinum electrodes on a pitch of 170 um. Assuming a production capability of 100 base pairs per site, these substrates could be capable of synthesizing oligonucleotides representing the entire Mycoplasma Genitalium genome [40].

**Figure 2.12** SEM micrographs of platinum grid GeneFab chips.

30
2.3 Photoelectrochemistry

After combining the photoconductive substrates and the electrochemical subsystems, a series of tests were conducted to determine if the expected acid generation contrast could be attained with this system. The photoelectrical system can be modeled for conceptual understanding by the equivalent circuit design in Figure 2.13, but the current-voltage characteristics of an electrochemical circuit are much more complicated to model than with these lumped circuit elements.

![Figure 2.13 An equivalent circuit model for conceptual understanding of the photoelectrochemical system.](image)

Photoelectrochemical activity was measured by placing a substrate in the three electrode fluidic and aligning the exposed glass window of the substrate to a 150 mW/cm² halogen lamp source. After injecting a 2 mM ferrocene solution in acetonitrile with 50 mM Bu4N+PF6−, cyclic voltammetry measurements were taken with the lamp on and off, and the results displayed in Figure 2.14. Clearly the photoconductive effect was capable of yielding the desired contrast in the oxidation and reduction of ferrocene. The experiment was repeated with a solution of 12.5 mM quinhydrone (equimolar amounts of hydroquinone and benzoquinone) in the 50 mM salt
solution, and a cyclic voltammogram was taken during exposure to light and displayed in figure 2.15. This unequivocally demonstrated that acid could be generated though photoelectrochemistry, as the CyV shows oxidation and reduction potentials of hydroquinone similar to those seen on platinum foil, with differences attributable to the Ohmic losses from conduction across the semiconductor.

**Figure 2.14** Photoelectrochemical oxidation of ferrocene.

**Figure 2.15** Photoelectrochemical oxidation of hydroquinone/benzoquinone.
2.4 Surface Chemistry for Oligonucleotide Linkage

Since the growing DNA is proximal to the electrochemistry during the deprotection step, it was rather difficult to devise an adequate linking chemistry that could withstand electrochemical processing, allow for reagents to diffuse properly, and adequately bind the DNA to the substrate. This linking chemistry can be broken down into two parts – first the development of an insulating micro-porous attachment layer bound to the surface of the chip, and secondly, the utilization of conventional organosilane linking chemistry that binds the DNA to the micro-porous reaction layer.

2.4.1 Porous Attachment Layer

In order to prevent the growing DNA strands from reacting electrochemically during the deprotection step, it was necessary to build an electrochemically stable membrane that could withstand the rigors of phosphoramidite synthesis for the attachment of DNA. This prevents the growing DNA from experiencing the extremely high electric fields generated at the electrode-electrolyte interface. Furthermore, by building the DNA on this membrane instead of at the surface, the short-lifetime radical and ion byproducts generated by the electrochemical reaction at the surface of the chip are less likely to have adverse effects on the growing DNA.

Several techniques are reported in the literature for forming porous membranes of various materials, including porous alumina through anodic oxidation of aluminum [41, 42] and several spin-coated, sintered sol-gel methods [32, 43]. These techniques form pores of various sizes, with aluminum anodization seemingly the most versatile method for arbitrarily controlling pore diameter [42]. The sol-gel chemistries follow relatively simple methods, but require high-temperature (>400C) sintering processes that could adversely affect the substrates. For this reason alumina as a porous reaction layer material was pursued prior to other less well developed techniques. After unsuccessful attempts at anodic and chemical oxidation of aluminum to generate a porous attachment layer, nanoparticle sintering based methods were attempted with freshly synthesized nanoparticles as well as purchased nanoparticles and precursors.

Sol-gel processes were investigated for the generation of a porous reaction layer because of the relatively low temperature processing temperatures involved in nanoparticle sintering [32, 44, 45]. Two different chemistries were attempted, one involving nanoparticle synthesis and
sintering and the other utilizing pre-made nanoparticle sintering. Although porous silica was preferred because of the ease of functionalizing a glass surface, titanium dioxide was also investigated because of its use in Graetzel type photoelectrochemical cells [32]. Porous titanium dioxide membranes were created via nanoparticle synthesis with titanium isopropoxide as a precursor, or by sintering solution dispersed nanoparticles purchased from Nanophase (Romeoville, IL). Porous silica layers were created via curing of Filmtronics Spin-on-Glass, via nanoparticle synthesis with tetraethoxyorthosilane (TEOS) and triethoxymethylsilane (TEMS), or with various nanoparticle dispersions made by Nissan Chemicals. Most of these methods were unsuccessful in generating the desired characteristics. All unsuccessful and suboptimal methods are listed in Appendix A.

Several dispersions of SnowTex silica nanoparticles from Nissan Chemical America Corporation (Houston, TX) were applied to glass slides. SnowTex ST-C, ST-PS-S, ST-UP and ST-OUP were tested, the properties of which are listed in Table 2.3. Each dispersion was applied to a glass slide at 2500 rpm for 30s. The slides were then put on a hot plate at 250 degrees under nitrogen, ramped to 400 degrees C in 30 minutes, left at 400C for 60 minutes, then cooled to room temperature over 60 minutes. All films but ST-UP and ST-OUP formed particulates on the surface of the glass and did not form any bonds to the glass surface after the high temperature step. Since Nissan Chemical lists all of the particle melting points to be greater than 500 C, the fact that the ST-UP and ST-OUP colloids formed sintered films was quite surprising – these films even passed a tape-test for adhesive strength.
<table>
<thead>
<tr>
<th>SnowTex Type</th>
<th>ST-C®</th>
<th>ST-PS-S®</th>
<th>ST-UP®</th>
<th>ST-OUP®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent</strong></td>
<td>H2O</td>
<td>IPA</td>
<td>H2O</td>
<td>H2O</td>
</tr>
<tr>
<td><strong>SiO₂ (wt%)</strong></td>
<td>20-21</td>
<td>19-20</td>
<td>20-21</td>
<td>15</td>
</tr>
<tr>
<td><strong>Na₂O (wt%)</strong></td>
<td>&lt; 0.2</td>
<td>&lt; 0.20</td>
<td>&lt; 0.35</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>8.5-9.0</td>
<td>9.0 - 10.5</td>
<td>9.0-10.5</td>
<td>2-4</td>
</tr>
<tr>
<td><strong>Particle Shape</strong></td>
<td>Spherical</td>
<td>String of Pearls</td>
<td>Elongated</td>
<td>Elongated</td>
</tr>
<tr>
<td><strong>Particle size (nm)</strong></td>
<td>10-20</td>
<td>10-15</td>
<td>9-15 x 40-300</td>
<td>9-15 x 40-300</td>
</tr>
<tr>
<td><strong>Viscosity (mPa.s.)</strong></td>
<td>&lt; 10</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
<td>&lt; 20</td>
</tr>
</tbody>
</table>

Table 2.3 Properties of various SnowTex dispersions tested (data from Nissan catalog).

It is hypothesized that the small particle diameter of these particles, or some impurities in the manufacturing process contribute to the low temperature sintering mechanism, but it is unclear exactly how. Since both ST-UP and ST-OUP sinter to form structurally stable films at these low sintering temperatures, and they are dispersed in rather different buffers, it is thought that the unique geometry of these particles contributes to the sintering temperature depression. Perhaps it is more surface contact or conformal packing that yields better heat conduction because of the cylindrical geometry of these particles – a long cylindrical particle with small diameter would have a much greater contact area to a flat surface than spherical particles, even though the surface area to volume ratio of the smaller, spherical particles is actually greater than the elongated particles. More analysis into this sintering process may yield exciting findings applicable to other nanoparticle sintering techniques.

As a test for porosity, ST-UP was applied and sintered on platinum covered glass slides to check for the electrochemical oxidation currents of ferrocene. The demonstrated current densities were comparable to a bare platinum surface. ST-UP was spun and sintered at the conditions aforementioned onto an n-type silicon wafer after a 3 minute argon plasma treatment on an n-type wafer. The argon treatment was used to improve surface wetting. A series of deep crevasses and an extremely rough surface are apparent in AFM (Figure 2.16(a)) and SEM (Figure 2.16(b) and 2.16(c)) images of the surface. Thus a new method for the low-temperature creation of porous glass films was created and utilized for the remainder of experiments.
Figure 2.16 (a) AFM micrograph of porous glass matrix on silicon made with SnowTex UP. Image was acquired on a DI Dimension 3000 AFM with 300 kHz tips using the commercial first order flatten algorithm. The scan rates are displayed in the legend.

Figure 2.16 (b,c) Same films viewed under SEM.
2.4.2 Organosilane Coupling Agents

The surface of a DNA microarray must have a suitable linking chemistry to attach a nascent strand of DNA and withstand the rigors of the phosphoramidite synthesis cycle. Silane functionalization is a widely used technique for DNA microarrays because of the various silane precursors available that are reactive with glass surface hydroxyls and the silane polymer stability after crosslinking [46]. Fortunately, glass is rather easy to functionalize with organo-silane linkers, although it has been demonstrated that silanization is possible with a variety of metal oxide surfaces, including titania, alumina and platinum black [47-53]. Generally, a silane precursor, such as a hydroxyl or amino functionalized chlorosilane or alkoxysilane, is reacted with the free hydroxyls on an oxide surface, and then permitted to cross polymerize with heat to form a resistant polysilane monolayer [54, 55]. It has been demonstrated that the stability of this layer is due to the silicon-oxygen-silicon bonds that form from condensation during heat treatment [54-56]. An idealized structure is shown in Figure 2.17, although it has been reported that most silane reactions create rather imperfect monolayers [57].

Custom Teflon substrate holders were designed and manufactured on the MIT Media Lab WaterJet (Omax, Kent WA) and put in Teflon containers from Savillex (Minnetonka, MN). Silanization was performed in a solution of 1% triethoxy-silane hydroxy-butyrate (Gelest Inc., Morrisville PA) in 95% ethanol [12]. Substrates were shaken gently at room temperature overnight. The substrates were rinsed four times with 95% ethanol and dried with nitrogen while heating to 50C to volatilize any remaining solvent. After the substrates were washed, they were placed in an oven for 2 hours at 100C under nitrogen to crosslink the silane layer. Substrates were slowly cooled under nitrogen to room temperature over 30 minutes. Substrates were always stored under nitrogen, which is particularly important for post silanization.
Figure 2.17 Idealized organosilane surface after functionalization with hydroxybutyramide triethoxysilane and heat induced condensation.

After silanization, the surface hydroxyls were protected with dimethoxytrityl. Dimethoxy trityl is used as a protecting agent for standard phosphoramidite synthesis and couples to free hydroxyls in a relatively simple Sn1 reaction. A gross excess of reagent was used to ensure adequate coupling. The solution contained 0.1M 4, 4’ dimethoxytrityl chloride and an equimolar amount of triethylamine as a scavenger in anhydrous pyridine. The solution was left to react for 4 hours on a shaker at room temperature.

Figure 2.18 Tritylated surface after reaction with dimethoxytrityl chloride.

Fluorescence microscopy was used to assay tritylation efficiency and verify deprotection. Since these initial tests were performed on a standard glass slide, the amount of free hydroxyls was assumed to be approximately equal to that of a single monolayer (as assumed for conceptual understanding in Figure 2.17 above). Fluorescence microscopy is one of the only imaging
techniques available for viewing a monolayer of dye in a pattern on a surface. Furthermore since future experiments would pursue fluorescent imaging, these initial experiments on glass helped to ensure that imaging covalent dye would function as an appropriate assay for reaction with surface hydroxyls.

After reacting a similarly silanized and tritylated glass surface, it was discovered by reaction with Rhodamine B isothiocyanate that the dimethoxytrityl reaction did not perform a perfect protection of surface hydroxyls. Rhodamine B isothiocyanate was reacted with the surface at a concentration of 1mM in dimethylformamide at 65 degrees C for 20 minutes. After viewing under a fluorescent microscope with appropriate fluorescent filters, intense fluorescence demonstrated that the dye was reacting with some free unprotected surface hydroxyls. Dimethoxytrityl groups were imperfectly covering the surface presumably because of steric hindrance, so an acetic anhydride capping step was introduced to completely eliminate free surface hydroxyls. The acetic anhydride capping step utilized the same reagents used during the phosphoramidite synthesis cycle - acetic anhydride with tetrahydrofuran in pyridine was combined with a solution of methyl imidazole in tetrahydrofuran at a ratio of 1:1, and reacted with the substrates for 5 minutes. As can be seen in Figure 2.19, this capping step reduced background reactions with free hydroxyls significantly.

In order to test for the expected surface protection and deprotection, and to verify that acetic anhydride capping was working as desired, three reaction conditions were applied to a single glass substrate. This was performed by adjusting the region of fluid contact to the substrate under different conditions. After silanization and tritylation, the substrate was placed in the fluidic cell in the first orientation. The first condition applied an acylating solution (acetic anhydride/methyl imidazole) was applied for 5 minutes, and then a mixture of 3% trichloroacetic acid in dichloromethane for 60 seconds. The substrate was repositioned so that a different area of the substrate would be

![Figure 2.19](image-url)
exposed to fluid contact, but that overlapped slightly with the first condition. In this case, the acylating solution (acetic anhydride/methyl imidazole) was applied for 5 minutes and rinsed. The third condition was the unmodified silanized, tritylated surface. Subsequently the substrate was contact printed for 120s with a PDMS stamp (soaked in 0.1 mM RITC, blown dry with nitrogen) at 65 degrees C and then thoroughly rinsed with dimethyl formamide. Contact stamping applies a conformal layer of dry molecules to the surface, which then react in the pattern that they were applied [58]. After contact stamping, the fluorescence microscopy image in Figure 2.20 was attained. Although all three sections show that dye reacted with the surface, it is clear that the detritylated surface showed a much higher surface fluorescence than the tritylated and acylated surface, while the tritylated-only surface demonstrated a strong fluorescence from uncapped reactive hydroxyls. Thus this surface chemistry for linkage and protection of the surface was demonstrated to suffice for assays for patterned photoelectrochemical acid deprotection.
2.5 Chemical Reagent Delivery

An ABI 394 Automated Synthesizer (394) was donated from the MIT Biopolymers Lab and served as the reagent manifold and delivery system. The synthesizer is capable of holding 19 reagents under Argon, and delivering a precisely controlled volume of reagent to a column or fluidic. The synthesizer is controlled by software written for the Macintosh and is capable of modifying, storing and editing reagent delivery cycles used to synthesize specific sequences. The synthesizer also has four relays that can trigger external events, such as turning on an external power supply, or send 4 bit messages to a nearby PC.

Since the DNA synthesizer typically uses standard column sizes, the standard DNA synthesis routine was altered in order to account for the volume of the electrochemical synthesis fluidic and associated tubing, which was precisely 1.2 mL. Additionally, I changed the cycle to include shuttling the new deprotection solution from one of the reagent reservoirs (reservoir 14, originally intended for ammonium hydroxide solution).
2.6 Maskless Lithography System

A maskless photolithography system was developed utilizing stock digital micromirror projectors and custom projection optics and mechanics. Patterns were generated on the substrate utilizing this maskless optics system. At first a highly modified Infocus X1 (Wilsonville, OR) projector was used to project light patterns onto the substrate, and then a newer, higher contrast and higher power Optoma EP719 (Milpitas, CA) projector. Since the platinum grids of the substrate needed to be aligned and focused to the light pattern from the projector, alignment optics and mechanics were also introduced. Between alignment and synthesis steps, it was important that the substrate maintained focus and alignment to the pattern, so a high power vacuum chuck was machined to hold the substrate in place.

![Optical Diagram](image)

**Figure 2.20.** Final optics for Infocus X1 projector. The Optoma projector optics were identical except that the UV filter and color wheel were in their stock positions rather than removed from the outputs of the lamp box and light tube, respectively (as above).

The X1 projector was modified to make it better suited for the purposes of this experiment, as seen in Figure 2.20. The UV filter on the output of the projection lamp and the color wheel were
removed to increase total optical output and the entire projection lens assembly after the DMD chip was removed. Although the best solution would be a complete restructuring of the output optics, due to mechanical constraints stock lenses were not easily adapted to capture all of the light from the DMD. I experimented with a simple 1:1 imaging system utilizing parts of the pre-condenser projector optics and two additional 90 mm focal length achromatic doublets from Melles Griot (Carlsbad, CA), but the system suffered from chromatic and spherical aberration, and did not collect light optimally from the DMD. As a replacement, a Rodenstock (Linos, Millford MA) Apo Rodagon D 1.2X enlarging lens was placed at the output of the projector, but this system suffered from coma, even after adjusting the built-in iris of the Rodenstock lens. Interestingly, utilizing a telescoping lens relay constructed from both of the achromatic doublets and the Apo Rodagon lens seemed to work the best. This system gave an aberration free field of view of 3.4x3.4 mm (corresponding to an array of 400 electrodes on the substrates). See Figure 2.21 for an example image of alignment with the platinum electrodes. With the projector at full power (white screen), the measured photon flux was 140mW/cm2 using a Molelectron Powermax 5100 laser power meter with a PM10 optical probe (Molelectron Detector, Inc., Portland, OR).

After initial experiments with the X1 projector, the Optoma EP719 projector was also utilized for experiments. This projector was unmodified except for the removal of the projection lens on the output. The projection optics were almost identical, except for the introduction of an iris between the APO Rhodagon D and the second 90 mm lens to alleviate coma. Since this projector boasts both an increased contrast ratio (2500:1) and higher output flux (1700 lumens) than the X1 (versus 2000:1 and 1000 respectively), it is a much better foundation for a maskless lithography system. The measured photon flux was 500 mW/cm2 without the iris.
Stage 1 controls zoom
Stage 2 controls focus of light onto substrate
Stage 3 controls alignment of substrate electrodes to DMD pixels
Stage 4 controls camera focus and image scroll
The gimble mount corrects for astigmatism

**Figure 2.22** Projection and alignment optics with substrate vacuum chuck for the Infocus X1 projector. The Optoma optics were modified to include an iris between L2 and L3, and the projector was mounted to the table, perpendicular to the optics rather than on the jack.

The projectors were mounted on an adjustable platform so as to allow image projection onto the substrate, while positioning the substrate and its fluidic proximal to the automated DNA synthesizer (Figure 2.22). A camera microscope was placed behind the substrate so that the light pattern could be focused and aligned to the substrate. The microscope utilized a 2X infinity conjugated objective lens attached to the camera with a complementary tube and tube lens.
2.7 Supporting Software for Controlled Patterning and Synthesizer Integration

Initial tests with the DMD were pursued by connecting the VGA input of the projector to a PC displaying a Microsoft PowerPoint presentation, thus determining which spots on the chip are illuminated. During each deprotection cycle, the projector outputs an array of appropriately located white boxes, which correspond to the sites at which photoelectrochemical deprotection is to occur. In the limiting case, these boxes would correspond to a single DMD pixel (15 um by 15 um), which when incident on the substrates tended to be around 40 um x 40 um due to optical dispersion from the silicon. Generally 1x1 or 2x2 pixel box sizes were used, separated by a pitch of around 13 pixels, to map directly to the electrodes on the surface of the substrates.

A custom Visual C# program was written to control all of the light and electrical inputs and outputs to the photoelectrochemical system. A Labview NIDAQ 6733E multifunction I/O board (Austin, TX) was controlled with the Labview Measurement Studio API's for Microsoft Visual Studio .NET, so that the input voltage to the potentiostat could be controlled, as well as measuring the current through the cell. On a system with dual monitors, a control screen on one monitor controls the image output to the projector (as the second DVI output). The software was capable of modulating the light or voltage controls to a user specification, so that for example, it was possible to alternate light and dark at 3 or less hertz, or alternate light to some pixels at a specified voltage, and then invert the pattern of light and set the potential of the chip at another specified voltage.

In this manner, it is possible to control acid and base generation at alternating sites to inhibit diffusion of acid, or even measure the current generated at an individual electrode. Thereby, the device could also function as a light addressable amperometric sensor if adequate signal to noise ratio could be attained.
Figure 2.23 Screen shot of control screen for projector control software.

Given a comma-delimited array of sequences in a text file, the software controls the output of the projector accordingly. The chip layout software changes the screen projected onto the substrate when triggered via a relay on the synthesizer. Additionally, the power source to the potentiostat can be switched via synthesizer relays to ensure that no electrical potential exists in solution during non-deprotection conditions. When a deprotection is initiated, the synthesizer relays activate the power source to the potentiostat and signal the computer to change slides to the next deprotection pattern. An automated light shutter was not implemented to shut off light completely during non-deprotection conditions, although it is recommended that future embodiments employ such a mechanism to completely shut off light to the substrate during non-deprotection conditions.
3.0 Results

3.1 Photoelectrochemical Trityl Deprotection and Phosphoramidite Coupling

In order to assay for deprotection, the fluidic was filled with the electrochemically deprotection solution, connected to the potentiostat circuit and placed in front of the projector. A variety of deprotection times and biases were attempted, starting from 1.8 V vs. the platinum reference electrode for 35s of light exposure. The initial voltage set point was determined by observing the cyclic voltammetry waveforms generated on the photoconductive substrates, and picking a potential that would be sure to oxidize hydroquinone under light exposure. The deprotection times and voltages were moved down as it became apparent that acid was generated very rapidly. Following the reaction, the reacted deprotection solution was immediately washed from the substrate with fresh deprotection solution, and a phosphoramidite coupling cycle was initiated on the automated DNA synthesizer. Cy3 phosphoramidite dye was used to assay for reaction with free surface hydroxyls. After deprotection and phosphoramidite coupling with a Cy3 phosphoramidite, the substrates were observed by eye and with a fluorescent microscope. Due to the incredible surface loading of the porous glass matrix on the surface, large patterns of dye were visible by eye under room lighting.

The first pattern was a grid of alternating 1mm light and dark spots, with a total surface coverage of 5 mm by 7 mm. After applying a bias of 1.8 V, and exposing the substrates for 35 seconds of 140 mW/cm2 white projector light from the X1 projector, and subsequent phosphoramidite coupling, it was clear that the substrates were already overexposed. The substrates demonstrated a semicircular dye pattern, with the arc of the circle formed by the o-ring in the fluidic and a line formed approximately at the bottom of the projected grid pattern. Since the reagent flow is from the bottom of the fluidic to the top, it is hypothesized that this line is a result of excessive acid generation and subsequent convection across the surface of the chip. Therefore, anything below the light pattern was thought to be acid free (hence no phosphoramidite coupling in that area), while everything above the light pattern was exposed to convected acid (hence phosphoramidite coupling). In order to correct this flaw, the exposure time was decreased to 10 seconds, and the applied bias was lowered to 1.7 V vs. the platinum reference electrode. Although in this case the grid was still not apparent, a box shaped outline at the location of the projected pattern was clear. Hence, the operating region required much less exposure time. Finally, a chopper at 10 hertz was placed at the output of the projector and the exposure time set to 5 seconds. In this case, a clear
grid with features 1mm by 1mm could be observed under fluorescence and by eye. This was the first ever demonstrated photoelectrochemically patterned phosphoramidite coupling.

Then, a grid with a one to one mapping of light pattern to array electrodes was attempted using the chopper at 10 hertz and a deprotection time of 5 seconds. Each electrode corresponded to four DMD pixels. After phosphoramidite coupling the image attained in Figure 3.1 was obtained. Although acid was reacting sub optimally, and convection was still an issue, at this point it was clear that with more optimization, photoelectrochemical arrays could indeed be synthesized at a very high resolution.

Figure 3.1 Fluorescent microscopy images of Cy3 phosphoramidite coupling after photoelectrochemical deprotection under 2x (left) and 5x (right) magnification.

The potential problems that resulted in poor pattern generation were identified as overexposure, lack of a buffer system for scavenging extraneous acid, stray projector light, convection of extraneous acid, and non homogenous illumination or electrochemistry. In subsequent experiments an organic base, triethylamine, was added to scavenge acid in solution. Stray projector light was diminished by replacing the Infocus X1 projector with a higher contrast, Optoma EP719 projector and an optical iris to lower the total amount of output light, down to 40 mW/cm2. Since it is reported in the literature that diffusion and convection problems with the electrochemically generated acid can be eliminated by proper design of the chemical buffer [59], no time was spent to develop better fluidic geometries or flow alteration. Although the software had been built to support inverting the image in order to generate base at alternating sites, it was hoped that the convection issue could be eliminated by eliminating extraneous acid through a good scavenging mechanism rather than methodological complications.

The next experiments were performed with essentially the same methods, but after replacing the X1 projector with the newer EP719 projector and adding 1mM triethylamine to the
electrochemical detritylation solution. Since the new projector had exceptional contrast, especially after blocking extraneous light with the iris, the chopper blade on the output of the projector was abandoned in lieu of alternating the projector light on and off through software control. The light was flashed on and off at 3 hertz for 5 seconds at a bias potential of 1.7 V vs. the platinum reference electrode. In this case, the alternating grid pattern generated was a little more regular across the total exposed area, though overexposure, convection, and other inhomogeneities were still a problem, as seen in Figure 3.2 (a-d). Nevertheless, at least part of the substrate shows clear contrasts in photoelectrochemically generated acid at resolutions of 100 um.

Figure 3.2 Deprotection with the Optoma projector for 5 seconds at 3 hertz with 1 mM triethylamine viewed with 2x (a), 5x (b), 10x (c) and 20x (d) objectives. The deprotection appears to be slightly more regular across the total exposed area than that under the previous conditions.

These patterns demonstrate the functionality of the maskless lithography system, the photoconductive devices, the electrochemical oxidation to yield acid, the porous attachment layer and associated surface chemistry, and the spatially selective detritylation reaction. In addition to
demonstrating the first spatially selective photoelectrochemical detritylation reaction, at least one other notable result is the stability and extraordinary surface loading of the porous glass layer.
4.0 Discussion

This technology required a significant amount of systems level integration in order to demonstrate the functional spatially selective detritylation reaction. Due to the number of variables in such a complex system, it was very difficult to address all of the variables that will yield an optimized photoelectrochemical array technology. Overall, every one of the supporting components could be optimized to provide a better synthesis platform. Nevertheless, this thesis does demonstrate that this technology is fundamentally capable of producing high density spatially selective chemical reactions and furthermore, phosphoramidite coupling.

The electrochemical instrumentation served well for these initial tests, but more advanced electrochemical designs should be pursued. For example, using a commercial potentiostat circuit could yield more precise measurements. Additionally, the hole made in the three electrode cell for the reference electrode should be eliminated to reduce heterogeneities in the electrochemistry, and the reference electrode made as a ring, or better yet, a platinum grid between platinum electrodes directly on the substrates. Although this would add a second patterning step to the substrates, it should be possible to turn them into a fully functioning amperometric or potentiometric light addressable sensor. In this manner, it would be quite easy to determine any heterogeneities in the light source or substrate design at the time of synthesis and make appropriate adjustments to the light exposure levels per pixel, or proper voltage settings. Furthermore, the light source could be modulated with specific voltage levels for specific spots if it were necessary, and if the array were turned into a sensor, drastically different types of electrochemistry could be driven by altering which spots are illuminated for a given set voltage.

The substrate design performed as desired in being electrochemically inert and generating the necessary photoelectric contrast ratio. Substrates could be used repetitively for electrochemistry without degradation. Even so, it was still greatly desired to test alternative designs of the substrate, such as utilizing a titania layer instead of patterned platinum electrodes. Removing the platinum patterning and high vacuum deposition steps would bring down substrate costs dramatically. It would also facilitate the addition of a platinum reference electrode directly on the chip because a mask alignment step could be eliminated that would otherwise be required with patterned platinum working electrodes.
The mask-less photolithography system would be much better off by completely replacing the projector optics. Since the projectors are meant to project images upward, when using part of the original projector optics, oblique light rays need to be corrected or eliminated, or the optical system suffers greatly from coma. This greatly reduces the useable area of the projected image. By replacing the entire optics of the projector, a perfect flat field 1:1 projection system could be attained and allow for individually addressing an entire array of 6000 or more electrodes.

The porous attachment layer that was disclosed in this thesis should be applicable to a variety of applications. Other microarray and solid phase synthesis technologies might benefit from this surface as it demonstrates an extremely high loading ratio per actual chip surface area. For example, achieving high yields of perfect DNA with photocleavable protecting groups is quite difficult – this optically clear attachment layer could help to improve the total amount of good DNA sequences produced in a given reaction. Upon further investigation, this matrix also withstands a 2-hour NoChromix (Godax, Cabin John, MD) soak in concentrated H2SO4, a process which obliterates any organic material, demonstrating the matrix’s stability and potential for recycling. Regardless of the potential applications, more research is needed to understand the mechanism of sintering of these particles, but it is postulated that the depressed sintering temperature is a result of their unique elongated geometry.

Clearly, the spatially selective acid generation is still in need of optimization. Given the sheer number of variables in optimizing this system and the limited time to try all possibilities, actually completing and verifying a photoelectrochemically patterned detritylation step posed many difficult challenges as it was. From the outset of these experiments it would have been wiser to attempt a smaller number of perfect detritylations on the array rather than initially attempting such a large exposure (400 pixels) array, so that perfect deprotection conditions could be found reliably.

The easiest operating region to adjust would be the set point bias potential. Since every experiment demonstrated overexposure, changing the bias potential to something closer to the oxidation potential of hydroquinone, or adding benzoquinone to adjust the equilibrium may allow for a longer and easier to control light exposure time. This adjustment of exposure time would lower the acid dose and most certainly generate better patterns. Additionally, there were many untested techniques that were hypothesized to generate better patterned surfaces. Although software was written to synchronize alternating light patterns with alternating potentials, in order
to alternate acid and base generation sites, this technique has yet to be attempted. This alternation strategy should certainly decrease diffusion of un-reacted acid. Furthermore, waiting for photoelectrochemically generated acid to react with scavenging molecules, while testing a variety of scavengers (such as other ternary amines, or pyridine or pyridine derivatives) and concentrations, should greatly reduce the amount of convected acid across the chip.

Although this project did not quite make it to the oligonucleotide synthesis level, several techniques were hypothesized that could make this a more suitable platform for gene synthesis techniques than other array technologies. Specifically, the potentially large amount of oligonucleotide that can be attached to the porous layer could allow for direct assembly of on-chip oligonucleotides rather than initially requiring amplification steps. Moreover, one difficulty in gene synthesis from chip is the amount of uncontrollable interaction effects after cleaving thousands of different nucleotides from the chip. This problem could be relieved by selectively cleaving oligonucleotides from the chip in pools via a photoelectrochemically generated base at the end of the cycle. Certainly this technology will undergo another round of optimization once oligonucleotides are routinely synthesized.
5.0 Conclusion

This project demonstrated the fabrication and validation of an inexpensive platform for microarray synthesis. A novel photoconductive substrate design for array synthesis was verified to have high contrast ratio photo-activated electrochemistry. Combined with the supporting mask-less photolithography system, light addressable spatially selective electrochemistry was verified.

This thesis also demonstrated a novel method for the low-temperature fabrication of a porous glass matrix for the attachment of biological molecules using cylindrically shaped SnowTex nanoparticle dispersions. Subsequent silanization and phosphoramidite coupling to this porous matrix demonstrate the extraordinarily high loading ratios as evident by the extreme fluorescence under room lighting of Cy3 phosphoramidite dye.

Lastly, this technique was used to generate local acid gradients to cleave dimethoxytrityl protecting groups from a solid phase support and selectively couple phosphoramidites dyes, delivered with an ABI 394 Automated DNA synthesizer with the phosphoramidite cycle conventionally used for DNA synthesis. At least part of these chips demonstrates detritylation directly above individual electrodes of size 100 um on a pitch of 170 um. As a comparison, the best commercially available electrically addressable Combimatrix 12k arrays have spot sizes of 45 um on a pitch of 75 um. Although actual DNA coupling has not been pursued, the Cy3 phosphoramidite coupling demonstrates this system’s merit for the potential synthesis of oligonucleotides, as there is little difference between additions of phosphoramidite dyes and phosphoramidite nucleotides. This system entails a complete photoelectrochemically addressable synthesis platform, including appropriate software control and systems integration, that should be capable of synthesizing customizable DNA arrays upon further optimization.

Compared to other available microarray technologies, this technology shows significant promise in producing the lowest cost high density customizable DNA microarrays to date. Furthermore, electrochemical reactions that can be light addressable also include radical initiations and electrochemically generated bases, demonstrating that this technology could be applicable to other types of combinatorial array synthesis, including peptide synthesis and polysaccharide synthesis. With optimization, this inexpensive light-addressable array synthesis technology has
the potential to yield the lowest cost custom oligonucleotide synthesis available, providing inexpensive oligonucleotides for genome scale engineering.
6.0 References

30. Tatsu Yoshinobu, e.a., Fabrication of Thin Film LAPS with Amorphous Silicon. 2004.


Appendix A.

Unsuccessful Attempts to Fabricate a Porous Attachment Layer

Porous Alumina through Anodic Oxidation

Alumina is stable under a range of chemical conditions, is capable of silanization, and is fairly stable mechanically [47, 49, 53]. Aluminum anodization yields a porous alumina layer with pore diameters determined by solution and anodization potential [42]. Aluminum anodization was attempted on glass slides coated with aluminum in a Teflon cell in 3% by volume sulfuric acid and a constant anodization current of 5 mA/cm². It was experimentally difficult to obtain the homogeneity reported in the literature, and thus this method was abandoned – the films generated were rather inhomogeneous, though clearly as demonstrated in the literature, more experimental determination of a good operating region should yield better films.

Porous Alumina through Chemical Oxidation

The idea of a porous alumina layer as a reaction layer was so attractive that another method was attempted through chemical oxidation. Five hundred nanometers of aluminum was deposited on the substrates. The substrates were immersed in a chemical solution of 0.2M iodine, tetrahydrofuran and water. After 3 minutes the substrates displayed an extremely dull, porous membrane instead of the previous shiny aluminum. Under SEM (Figure A.1), the aluminum had clearly oxidized into a rough, possibly porous surface. However, post oxidation substrates did not show any photoelectrochemistry – a probable result of a barrier oxide layer of alumina directly on the platinum electrodes. It has been reported in the literature that the barrier oxide could be removed by reverse biasing the substrates in phosphoric acid [41]. After immersion of the substrates into 3% phosphoric acid for 3 minutes, or reverse biasing the substrates at -3V during 150 mW/cm² illumination for 1 minute, the substrates still did not yield any electrochemistry. Since the foray into aluminum oxidation was yielding improper films, other techniques were investigated that required less resources for development.
Titanium Dioxide from Synthesized Nanoparticle Precursors

Titanium dioxide nanoparticles were synthesized by reaction of titanium isopropoxide precursors in an ethanol solution with acetic acid as a precursor [60]. This solution was composed of a molar ratio of 1:9:0.1 titanium isopropoxide:ethanol:glacial acetic acid. This solution was spun on gold slides at 2500 rpm for 30s and baked at 250°C in air. These films were mechanically and chemically stable. Although these films yielded the expected oxidation-reduction peaks for ferrocene, the current density was 1/100 of the current density on bare gold – most likely a result of insufficient porosity or a few defects in an otherwise perfect film. As a result, no further exploration of this technique was pursued.

Titanium Dioxide from Suspension of Purchased Nanoparticles

Titanium dioxide nanoparticles from Nanophase were dispersed in deionized water at a concentration of 10% by weight and then filtered through a 1μm pore syringe filter. Although the particles are reported to be on the order of 35 nanometers, they could not be filtered.
through a 0.4 um syringe filter. This could be a result of some kind particle instability or aggregation, as these particles were several years old. Five hundred microliters of this solution was deposited on a platinum covered glass substrate and spun at 2500 rpm for 30 seconds. The substrates were put on a hot plate at 250 degrees under nitrogen, ramped to 400 degrees C in 30 minutes, left at 400C for 60 minutes, then cooled to room temperature over 60 minutes.

The films could not be pried off with VWR tape, demonstrating that they were stable enough to be a suitable solid synthesis support. Furthermore, a cyclic voltammogram could be obtained demonstrating oxidation/reduction current densities comparable to a bare gold surface. Although this surface could be sufficient as a porous matrix for synthesis, the number of large particles on the surface after sintering seemed sub-optimal, and the ease of silanization of porous glass compared to titanium dioxide ushered experiments into the development of low temperature porous glass films.

**Silicon Dioxide from Spin on Glass**

Filmtronics 15A (Butler, PA), a silica spin on glass dielectric, was applied to gold slides with the hopes that a slightly porous silica membrane could be obtained at low cure temperatures. The solution was allowed to warm to room temperature overnight and then spun on the slide at 3000 rpm for 15s. After curing at 250C for 1 hour in an oven, there was absolutely no noticeable electrochemical current from the oxidation or reduction of ferrocene, demonstrating a very high density film.

**Silicon Dioxide from Two Phase Nanoparticle Colloid**

A two-phase silica nanoparticle colloid was synthesized with tetraethylorthosilane and triethoxymethylsilane as the precursors according to Makita et al. who demonstrated porous silica membranes with regular pore sizes up to 100nm with this method [43]. These films were produced with a ratio of 3.5:1 tetraethylorthosilane to triethoxymethylsilane. These films demonstrated weak electrochemical current densities. After spinning on an n-type silicon wafer and curing at 250C for one hour, the AFM image in Figure A.3 was obtained, demonstrating the sparse porosity of this film. Although this technique did not generate sufficient porosity as attempted, the authors report superior porosity than that witnessed in the AFM image, and it is likely that experimental optimization would yield the desired porosity.
Figure A.3 Porous glass structure made from TEOS and TEMS precursors.