Visualizing Amine Groups Tethered to a Glass Surface

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Abstract

This study determined the viability of a new technique for visualizing micron (or smaller) sized areas of amines tethered to a glass substrate. The experiment utilized a methodology that requires the use of amine functionalization, plasma cleaning, and an elastomeric mold to pattern amines on a glass surface. Electro-less copper metallization was used to verify that the patterned amine region was indeed present on the surface of the glass. The results suggest that metallization can be used to detect amine groups on glass; however, more experimentation is needed to attain maximum selectivity in the devised visualization technique.

Introduction

In science, advancement is essential. If scientists can develop better technology to build and process microarrays then the advancement in biotechnologies can be conceptually limitless. Previously, the field of genomics, the field of chronicling genomes of various species, was limited by the lack of technology. This caused the first part of the Human Genome project to take years to complete. Today, a scan of the entire human genome can take a few minutes and rebuilding the genome can take less than a week to complete. The availability of this technology has vastly improved the ability of scientists in the field of disease prevention to be able to pinpoint genetic markers for various illnesses. Similar to the computer revolution in which computer processing improved in power and speed by reducing the size of computer chips, a new direction in genomics has been fueled by microarrays (Schena, 2003). By further reducing the size of microarrays to the nano or micro-scale, technology in the biotechnology fields will be improved, and this improvement will lead to more understanding and efficiency in health fields and disease prevention.

Schena (2003) defines the term microarrays as a "scientific word derived from the Greek word *mikro* (small) and the French word *arayer* (arranged)" (p 4). More simply put, microarrays consist of arranged fragmented DNA or protein pieces attached to a substrate or ligand. These devices are used as diagnostic and analytical tools in the fields of biotechnology and genetics. The first step in the actual creation

of microarrays is to functionalize (which is to place groups of compounds on) the surface of the substrate. In the second step, DNA is placed on the surface of the ligand, usually by a printing technique controlled by a mechanical system. The functionalizing compounds, usually amino-silanes or epoxy-slianes, are used to attract and hold the strands of DNA electrostatically or covalently to the surface of the substrate (Blalock, 2003; Sheena, 2003).

Many techniques exist to confirm that amine groups are on the surface of the substrate. (Moon et al, 2007). These techniques are helpful to confirm that the amine groups are present in the desired arrangement before analyzing DNA microarrays. Selective metallization is a good technique because of its simplicity and the ease of obtaining good visible results. There appears to be no reported studies using this method to detect micron sized areas of amine on glass substrates.

The purpose of the study is to determine if metallization (electro-less deposition) can be used as a viable technique to detect micron-sized (or less) areas of amines patterned on a glass substrate. The goal is to provide a relatively simple method to confirm a desired pattern of amines on glass. The glass will be patterned by selectively cleaning away certain areas of an amine functionalized substrate. This selective cleaning will be performed by protecting specific areas of amines using a polydimethylsiloxane (PDMS) stamp. Questions to be addressed include:

- How necessary are amine groups to metallization?
- How effective is the plasma cleaning or etching in removing amine groups during selective cleaning?
- What are some of the variables that can be adjusted to improve the selectivity of the metallization process?

Background and Research Plan

Background Terms

Microarrays. Microarrays consist of pieces of fragment DNA or protein attached to a substrate or ligand (the medium to which the DNA fragments are attached to). These arrays are used as diagnostic and analytical tools in the fields of biotechnology and genetics. Microarrays are essential in chronicling genetic material and, forming gene libraries, such as the Human Genome Project. For the last few years, microarrays have gained importance as a technique to understand the frequency with which genes are expressed for various species. The development of microarray technology leads to more understanding of gene expression which has vast applications in medical technology, forensic evidence, pharmaceutical production, environmental restoration, and agricultural advancements (Blalock, 2003; Reece, 2004; Schena, 2003; Schena, 2005; U.S. Department of Energy Office of Science, 2008). For instance, understanding of gene expression, which is the process by which genetic information flows from gene to messenger RNA and is converted to a protein (the state of a gene being activated), has led to the development of gene therapy (Reece, 2004; Schena, 2003). Gene therapy is the ability to repair or

replace faulty genes with a properly functioning gene. However, gene therapy has been stalled because the expression of one gene can influence the rate or intensity in which other genes are expressed.

While microarray technology and bioinformatics adds valuable knowledge to unexplored areas of biology and genetics through the use of supercomputers, the data generated is so vast that the ability to analyze and reproduce the data is very limited and unreliable. According to Blalock (2003), "Regardless of the application, generating high quality microarray data requires reliable manufacturing and processing techniques" (p 94). Each microarray manufacturing company has to deal with specific variables within their production technology; no production system will be exactly the same. Troubleshooting for each manufacturing company is necessary. Even if a standard methodology for creating microarrays existed, each machine (microarrayer) would contain nuisances and variables specific to that machine that would need to be controlled. Simply stated in Blalock (2003), microarray manufacturing is a science in which the user tries to control variables in their system to generate a reliable and reproduce product. The variance in the production and manufacturing of microarrays leads to technological variance which has to be over come in addition to the experimental variance of the biological systems. These technological variances limit the reliability and reproducibility of the microarray products, which means that the development of new technologies to manufacture and analyze microarrays could be the break through that could cause a revolution in not only health care and medicine, but would also advance biotechnology in a way that would change the concept of ordinary everyday life. Picture a future where a swipe of a customer's finger could instantly put the charges on his or her bank statement, where drugs can be genetically tailored to fit an individual's genetic material, and where medical diagnoses would be more efficient and accurate.

Manufacturing of Microarrays. Microarrays are made using sophisticated robotic systems, arrayers, in order to mass produce consistent microarrays at a small cost. The first step in the actual creation of microarrays is to functionalize, or place groups of compounds on, a surface of the substrate. In the second step, DNA is placed on the surface of the ligand usually by a printing technique controlled by the mechanical system. The functionalizing compounds, usually amino-silanes or epoxy-slianes, are used to attract and hold the strands of DNA electrostatically to the surface of the substrate (Blalock, 2003; Sheena, 2003).

Although, glass is an ideal substrate because of "the combination of structural roughness and highly polar surface groups," the polarizable groups on the surface of the glass allow for environmental contaminants such as organic molecules and debris to attach to the glass surface (Blalock, 2003). These surface contaminants prevent the amine groups from adhering to the polarized groups, and this prevention causes the amine coating to be non-uniform. Thus, before any substances are placed on the substrate, the substrate must be sterilized to rid the surface of contaminants. This research paper will explore two viable to techniques to clean surfaces. The first method exposes the glass surface to oxygen plasma, and the second method

involves the use of Hydrofluoric acid (HF). Although both methods of sterilization function in a different ways, each technique produces hydroxyl groups bound to the silicon atoms at the glass surface.

Plasma Cleaning. Plasma cleaning is an essential element of the amine functionalization or slide coating process. Plasma cleaning is gaining popularity in the slide coating process because this technique eliminates organic contaminants without the toxic waste generated in an acid or alkaline cleaning process. Oxygen plasma cleaning functions by stimulating oxygen plasma (a substance that contains properties of a gas and liquids) through low or high frequency radio waves in an oxygenated environment. These radio frequency waves cause diatomic oxygen molecules to split and become extremely reactive. When these oxygen atoms come into contact with surface contaminants, the molecules are oxidized into water vapor, carbon monoxide and carbon dioxide. These gases are removed by the vacuum stream (Birch, 2003; Blalock, 2003).

Hydrofluoric Acid (HF). The use of HF is one of the oldest methods to clean glass. However, the chemical reactivity of HF makes it extremely dangerous. HF is a powerful acid that has the ability to decalcify bones and cause lung and heart failure. HF operates by literally eating away the surface of the glass and any contaminants on the surface. According to Birch (2003), "The fluoride ions rupture siloxane bonds" (Alkaline solution cleaning, paragraph 3). The result is that pieces of glass break away from the surface, and this removal of glass causes the surface of the glass to become rough. The amount of etching or cleaning required depends on the concentration of HF and the amount of time the substrate is in contact with acid.

Amine Coating. By manipulating the amine coating through patterning, scientists can validate the effectiveness of their patterning technique before the manufacturing of their microarrays. Amine groups are not the only compounds used to functionalize glass. Epoxy groups and aldehydes are also commonly used. However, the aldehyde coatings can sometimes create bias in microarray analysis due their hydrophobic interactions (Schena, 2003). The amine groups are functionalized to the glass surface with the use of amino-silanes. Aminosilanes are a group of molecules containing amine groups (which are groups of atoms with a nitrogen atom possessing a lone pair of electrons) bonded to a central silicon atom. The aminosilane compounds react to the surface of the glass, which is composed of silanol groups. This reaction allows the aminosilane groups to attach to the surface (Blalock, 2003; Schena, 2003). The amine groups can then react with the phosphorus atoms of the DNA and binds the DNA strands to the substrate forming microarrays. Conversely, these amine groups can be used to deposit metal onto a substrate, and by doing so they essentially confirm their presence on a substrate.

Selective Metallization. Selective metallization is a process to distinguish the shape or patterning of a compound by depositing metals onto its surfaces. Usually, the metallization process is used to distinguish the amine groups of a compound or to confirm that the amine groups are present in the desired arrangement before conducting DNA analysis. However, many types of selective metallization exist.

New methods of metallization and photolithography are constantly evolving and being created especially for applications concerning circuit boards.

Traditional methods. Early methods of metallization were based solely on the concepts of etching and lithography (Ferrier & Larson, 1990). In print making processes such as etching and lithography, metal is removed to create a design. The difference between the two art techniques, lithography and etching, is the way in which the removed area is used to create either a negative pattern or positive pattern. Rathus (2007) explains that in some print making processes the, "Areas that are not meant to be printed are cut below the surface and, areas that are meant to be printed are left raised" (p146). Another print making category, which includes etching, uses the depressed or removed surfaces of the metal to form the design (Rathus, 2007). The elements that separate etching and lithography from other print making processes are the use of chemicals to either remove metal or precondition the surface of the metal for manipulation.

Ferrier and Larson (1990) described the early methods of metallization as either subtractive or additive. According to Ferrier and Larson (1990), "[the characteristic] common to the subtractive process is the need to etch away (or subtract) metal to expose substrate surface in areas where no circuitry is desired" (paragraph 8). The flaw of this technique was that it produced error in the results of the metallization process by exposing some areas of the substrate that should not be exposed. Also, the technique was not economical because it discarded metal material which has to be re-processed to be reused. Ferrier and Larson (1990) noted that, "Additive processes, on the other hand, begin with exposed substrate surfaces and build up thereon metallization in desired areas, the desired areas being those not masked by a previously-applied pattern of plating resist material …" (paragraph 10). Flaws of the additive process included limited choice of metal material and ability to build the metal coating to the desired specification.

The difficulties of the additive and subtractive processes led to the creation of a hybrid method of metallization which included methods from both processes. In the reformed process, the surface of the chosen material, called the substrate, was first coated with a layer of electro-less metal and then areas that metallization was unwanted (resist) were stripped away or etched off to reveal a metalized pattern. Ferrier and Larson (1990) explained that, "In this process, however, the deposition of metal over the resist is quite substantial and leads to difficulties in cleanly stripping the resist, often resulting in the remaining metallized areas adjacent the resist (e.g. conductors) having ragged edges or slivers, correspondingly poor fine line resolution or definition, and risk of shorting" (paragraph 12). The weaknesses of the hybrid process lead to the modernization of selective metallization which required a systematic use of solvents to induce the coating of the substrate.

Modern methods. Most modern electro-less metallization techniques use a three step method that calls for a preparatory agent, followed by a catalyst immersed in some type of activating and/or deactivating agent, and in the final step the substrate becomes metalized (Ferrier& Larson, 1990; Charbonnier, Romand, Harry, & Alami,

2001). However, many methods combine two of the three steps to make a more economical step (Ferrier& Larson, 1990). The preparatory step, also referred to as the preconditioning phase, functions by blanketing the substrate surface in a thin film, which allows an increased surface for metal deposition to take place; however the mechanisms for the adhesion of the metal to the preparatory agent is not full known (Dressick & Calvert, 1993; Ferrier& Larson, 1990). The catalyst covers the substrate in seeds of positively charged ions or catatonic metal molecules which attract the metal that will be deposited.

The process used in the study was adapted from the work of Charbonnier et al because of the simplicity and clarity in the procedures. The procedures require plasma cleaning for the first step followed by the addition the catalytic $PdCl_2$. Then the substrate is exposed to a reducing bath, and then to a copper solution mixed with formaldehyde (which induces metallization). The complete process is described in the methodology section of this report.

Research Plan

The plasma cleaning technique had dual functions in this study. The first function is associated with the creation of the amine functionalized slides, and the second function is to form a pattern by etching away unwanted compounds for the substrate. Knowing the chemical nature of oxygen plasma allows scientists to manipulate the characteristic of the plasma cleaning process to pattern amines through the use of a protective mold. The protection stamp or mold is created by a substance called PDMS (Polydimethylsiloxane).

PDMS is an elastomeric polymer with the ability to form an air tight seal on the glass substrate. This seal prevents the area underneath from being exposed to the reactive oxygen atoms. The results are that unwanted (unprotected) amines are etched off the surface of the substrate leaving only the areas where the mold was sealed to the glass. The existence of a patterned amine slide, however, is still an assumption that must be proven by some technique or spectroscopy. The only way to prove that the amines are arranged in the desired pattern is by employing a technique to visualize (see) the pattern. The flaw of spectroscopy, such as XPS (X-ray Photoelectron Spectroscopy) is that the technique only proves that amine groups lie on the surface of the substrate, but it can not prove that the amine groups form a pattern. So the technique presented to confirm that the substrate was pattern in a pre-determined design was metallization because it is simple and cost effective.

Methodology

Palladium Solution. To create the palladium solution about 0.01 grams of palladium chloride ($PdCl_2$) was placed in 100 ml of de-ionized water with stirring until the solution turned to a pale yellow color. The solution was then processed through 0.65 micron filter.

Copper Solution. The reagents Copper sulfate pentahydrate (0.28 M Cu SO_4 * $5H_2O$), sodium tartrate tetrahydrate (0.60 M $KNaC_4H_4O_6$ * 4 H_2O), 0.60g ethylenediamine tetraacetic acid (EDTA) were mixed in 5 ml of deionized water. Sodium pellets were added to increase the pH to about 11. The solution was filter through 0.2 micron filter.

Amine Coating. For the study, #2 glass cover slips were used as the substrates. The substrates were sterilized then coated in an amine solution consisting of 5 ml aminopropyltrimethoxysilane in 100 ml of methanol for four hours. Following the functionalization the substrates were sonicated then dried for a few minutes in the oven.

Metallization. The substrate with a PDMS protection stamp sealed to its surface was placed in the plasma cleaner to remove unwanted compounds from exposed areas. The PDMS stamp was removed and the substrate was placed in a beaker of palladium solution for about 15 minutes. The slide was washed thoroughly, rinsed in three washes of deionized water, and then placed in a reducing bath of $NaHPO_3$ (0.1 M Na HPO_3 * H_2O) at a temperature of 84°C for about 10 minutes. The substrate was then rinsed for a minute and left to air dry. Then the copper solution was mixed according to the ratio of one part formaldehyde to five parts copper solution. A few drops of the copper and formaldehyde mixture were dripped on to the surface and time was allotted for metallization to occur. After a sufficient time, the substrate was rinsed one final time to remove any excess copper and the copper solution.

Results

In order to determine the viability of the metallization process, one amine functionalized slide was plasma cleaned with a PDMS stamp in place. The results appeared to convey (Figure 1(a)) that metallization was not a specific method because more metal occurred on the sides of the substrate rather than on the desired area (middle). However, that slide (shown in Figure 1(a)) exhibited hydrophobic interactions in the middle of the slide, and this caused the copper and formaldehyde mixture to flow more readily to the areas at the sides of the glass. The sides of the glass therefore had a higher probability to metalize because the copper solution was more prevalent around the edges of the substrate.

To test if amine groups were necessary for metallization, an as received glass slide was subjected to the metallization process. For this slide, metallization was centralized in the middle, where the PDMS had been positioned during plasma cleaning. However, substantial metallization also took place on the edges of this slide. The conclusion derived from this sample was that maybe metallization was not a very selective process or that maybe some contaminants were still present on the edges of the substrate even after the plasma cleaning.

Figure 1 (a)

Figure 1 (b)

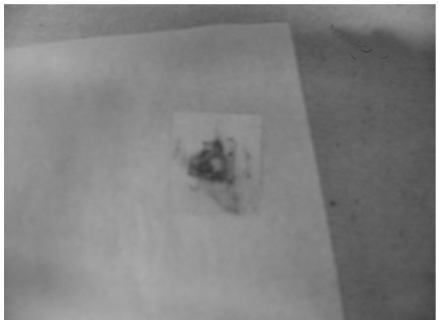

Figure 1: (a) An amine functionalized slide that was selectively cleaned using a PDMS stamp. More metallization occurred on the sides of the slide than in the middle of the slide where the protection stamp was placed. (b) A functionalized slide with the protected area in the center of the slide, the most of the metallization occurred on the protected area. The hole that appears in middle of the metalized occurred because the PDMS piece was not sealed to the glass in that spot.

To determine if metallization in the unprotected region was due to contaminants, the plasma cleaning time was increased to 6 minutes. Two functionalized slides were used. One was pre-rinsed and the other was not. No PDMS stamp was utilized for any of these slides. Although both slides were metalized following the same metallization procedure, one slide experienced more metallization than other (Figure 2). The lack of metallization in the center of both slides suggested that contaminants were mostly cleaned from these areas. The sides of the substrate could have been contaminated after the plasma cleaning because the slides are handled by holding the sides of the slides. However, if the slides were contaminated due to handling then the non-coated slides that were plasma cleaned would have contained metallization along the sides (not shown). These non-coated slides showed no such metallization, and so it is currently unclear why the sides of these substrates were metalized. Nevertheless, the lack of any metal deposition near the slide center suggested that increasing the plasma cleaning time helped to reduce the amount of surface contaminants and any undesired amine groups.

Figure 2 (a)

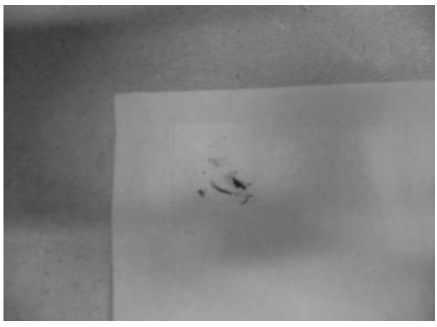

Figure 2 (b)

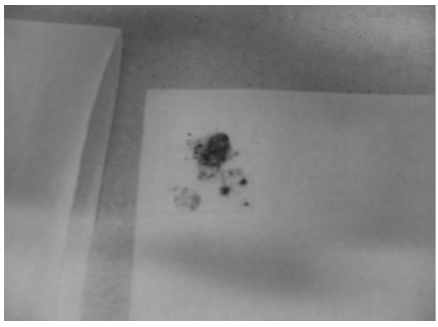

Figure 2: (a) A functionalized slide that was pre-rinsed in methanol and acetone, and was plasma cleaned for 6 minutes. Metallization did not confirm the predetermined pattern. The metallization observed was not concentrated in the middle of the slide. (b) An amine functionalized slide that was pre-rinsed in methanol and acetone and plasma cleaned for 6 minutes. Metallization occurred all over the slide; however, the metallization might be due to the temperature of the $NaHPO_3$ being lower than normal.

By understanding that more time is required to clean functionalized substrates, the next experiments were devised to test the optimum time for a functionalized substrate to be cleaned and a clamp's effect on the selective cleaning process. The clamp was used to hold the PDMS stamp against the glass substrate. The idea being that this would create a better seal between the PDMS and glass. It was predicted that more time would be needed to clean the unprotected areas of the substrate because the clamp would reduce the amount of plasma that would come in contact the surface of the substrate.

First, two functionalized glass slide were cleaned for 12 minutes with no PDMS stamp or clamp. The metallization of one substrates showed copper deposition only along the left edge (Figure 3(a)). The other slide was pre-rinsed in methanol and acetone before it was plasma cleaned which resulted in a clean slide. Another slide (not shown) was subjected to the same variables with the exception of a slightly longer cleaning time, and similar results were achieved. The results (Figure 3(b)) indicated that plasma cleaning was suitable enough to clean a functionalized slide. Thus, it was probable that more time would be needed to clean a functionalized slide placed in a clamp because the clamp would prevent some of the oxygen plasma from reaching the glass surface.

Two slides were used to test this idea. The time for the plasma cleaning was chosen to be 25 minutes. The results showed a concentration of metallization in the center of the substrates (Figures 3(c) and 3(d)). However, metal also appeared in unprotected areas, which suggests that not all amines were being cleaned off unprotected areas or that maybe hydroxyl groups were also experiencing metallization.

Figure 3 (a)

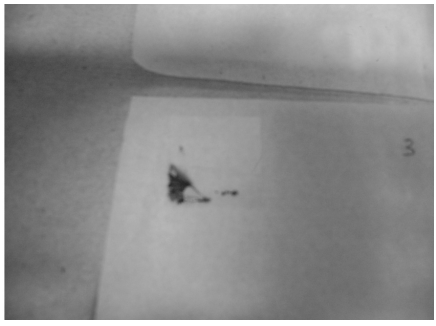

Figure 3 (b)

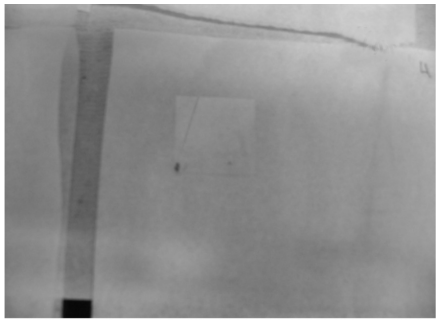

Figure 3 (c)

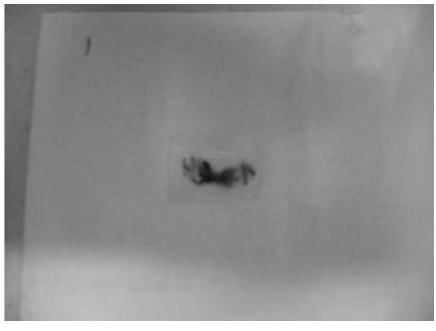

Figure 3 (d)

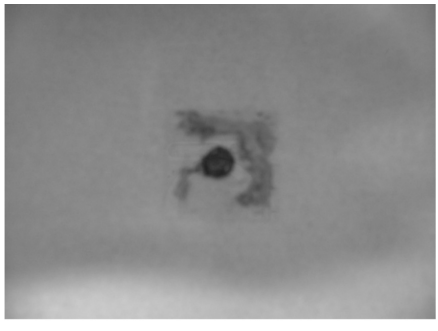

Figure 3: (a) A functionalized slide, that was not pre-rinsed in methanol and acetone was used. The substrate was plasma cleaned for 12 minutes. Metallization occurred extensively on the bottom corner of the substrate. (b) The slide appearing here is an amine functionalized slide that was pre-rinsed in methanol and acetone, plasma cleaned for 12 minutes. Small specks of metallization occurred on the slides but the metallization was not significant. (c) Shown is a functionalized slide that was pre-rinsed in methanol and acetone, and plasma cleaned for 25 minutes. No PMDS stamp was used; however the clamp was placed around the slide. (d) This functionalized slide was pre-rinsed in methanol and acetone and plasma cleaned for 25 minutes. A PDMS stamp was used to protect the desired area from being plasma cleaned. Metallization occurred more extensively in the desired areas, but metallization still occurred on the sides of the substrate.

To verify or eliminate the possibility that hydroxyl groups were causing metallization, a functionalized slide that had been selectively cleaned using a PDMS stamp was exposed to vapor of a fluorinated silane, (tridecafluoro-1,1,2,2-tetrahydrooctyl) dimethyl chlorosilane. The proposed plan was that fluorinated silane molecules would react with the hydroxyl groups on the surface of the glass preventing any metallization by these hydroxyl groups. For this sample, the large

contact angle for a drop of the copper-formaldehyde mixture on the glass substrate suggested that the fluorinated silanes were on the surface of the substrate. But metallization still occurred in unprotected areas, and this confirmed that amines groups were not being completely removed from exposed areas.

These amine contaminants could also be blamed for metallization occurring outside the protected area of amines. The standard procedure for plasma cleaning usually calls for materials to be sonicated first. So two slides were sonicated for five minute intervals in acetone, isopropyl alcohol, and water, and then baked to dry. For these samples, metallization was concentrated in the middle of the slide, but unexposed areas still metalized (Figures 4(a) and 4(b)).

It was proposed that maybe the hydrophobic interactions influenced the how the substrate metalized; therefore the ratio of formaldehyde to copper solution was increased (from 1:5 to 1:1) so that all of the substrate would be covered by the copper solution at the same time. The effect of changing this ratio led to no metallization occurring on the slide at all.

Figure 4 (a)

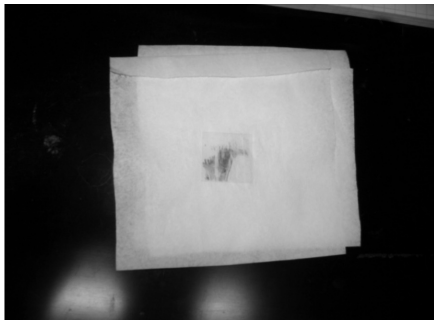

Figure 4 (b)

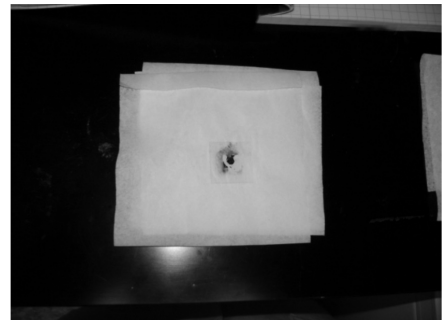

Figure 4: (a) This functionalized slide was sonicated for 5 minutes in sequential washes of acetone, isopropyl alcohol, and a water rinse. The slide was dried in an oven at 150°C for ten minutes. While the metal is denser in the desired area, pockets of metallization still took place along the edges of the cover slip. (b) This slide (functionalized) was also sonicated for 5 minutes in sequential washes of acetone, isopropyl alcohol, and a water rinse. The slide was dried in an oven at 150°C for ten minutes. Then the slide was metalized and the observed result was that while metallization mainly occurred in the middle of the slide, and metal still appeared on the sides of the slide.

Another possible process to clean unwanted amines off the surface of the glass was to use HF. HF literally removes glass layers from the surface of the substrate. Therefore, any amines in exposed areas of the substrate would be removed during the selective cleaning step because that area of glass substrate would be eaten away, dislodging the amine groups in these areas. The results reveal that metallization appeared everywhere, suggesting that HF did not remove the unprotected amine groups from the surface of the glass (Figures 5(a) and 5(b)).

Figure 5 (a)

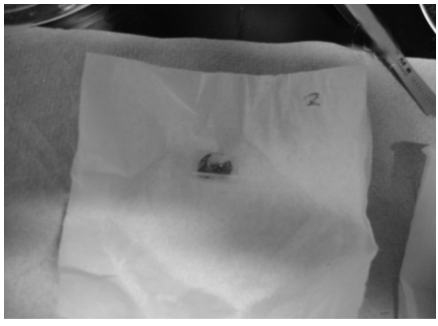

Figure 5 (b)

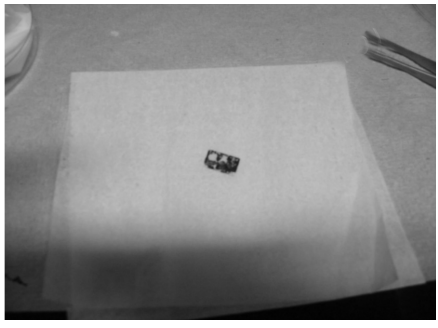

Figure 5: (a) This amine coated slide was subjected to HF for 30 seconds; the slide was metalized and metal deposited on the entire slide. Some of the metal had peeled off during the rinsing. (b) The functionalized slide shown was placed in HF for two 10 seconds dips to determine the viability of the HF etching for the metallization technique. The result observed was that the entire slide metalized. The holes that appear on the slide are the result of the copper solution peeling off during the rinsing.

Another strategy tested was to functionalize a PDMS stamp and place it on a plasma cleaned slide. A non-functionalized slide that has been plasma cleaned will not metalize (not shown). Thus, the plan was to use a PDMS stamp to place amines on selected areas of a clean slide. This stamping process would avoid problems associated with trying to remove amine groups from certain areas of a slide. However, removing the stamp from the cleaned slide after 2 hours and exposing the substrate to the metallization solutions led to the metal to deposit everywhere on the slide (Figure 6). The proposed explanation is that amine groups radiated out from the location where the stamp was placed to the fringes of the glass slide. It is plausible that a shorter stamping time, using an amine functionalized PDMS stamp, will produce better results.

Figure 6

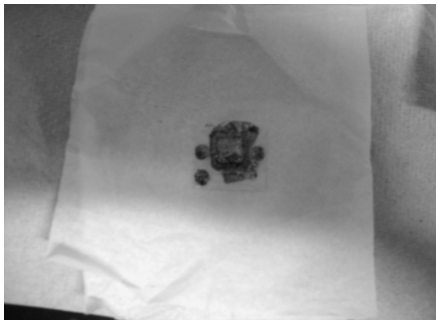

Figure 6: Displayed is a non-functionalized slide that had a functionalized PDMS stamp (which had been exposed to the amine solution (10 % v/v APTES in methanol)) placed on its surface for 2 hours. The slide was then metalized. Metallization occurred all over the surface of the slide, and the metallization pattern seemed to contain swirls.

Another proposed strategy to deal with the occurrence of metallization in unexposed areas involved reducing the palladium concentration in the seeding solution. A palladium concentration that is too large may blanket the entire slide with palladium instead of limiting the palladium to the protected amine groups. A slide was exposed to the reduced concentration palladium solution, and then exposed to the other metalizing solutions. Metallization occurred all over the surface of the slide (Figure 7(a)). The results from this slide suggested that while the amine groups in the protected areas receive more deposits of palladium than the unprotected, plasma cleaned areas of a slide (and therefore react quicker with the copper solution), exposing these unprotected areas to the copper solution for long times causes metallization in these areas.

So a time limit was proposed for the substrate's exposure to the copper solution. Another slide (Figure 7(b)) was also introduced to the reduced concentration of palladium; however the exposure to the copper solution was limited. This strategy produced the best results (Figure 7(b)). When the same variables were repeated (see Figure 7(c)) the results were not similar to metallization pattern of Figure 7(b). It is believed that an ineffective stamp caused the variability. The proposed explanation was that stamp used for this particular substrate had lost its adhesive ability due to contaminants on the surface of PDMS. The next step was to try to plasma clean the PDMS stamp. However, the plasma clean caused the stamp to lose its adhesive ability all together.

Figure 7 (a)

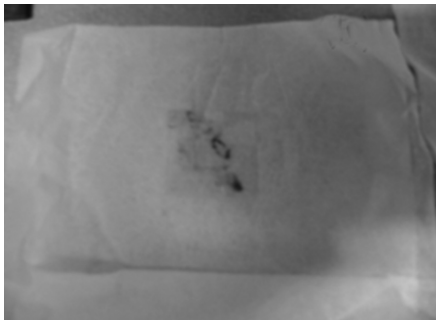

Figure 7 (b)

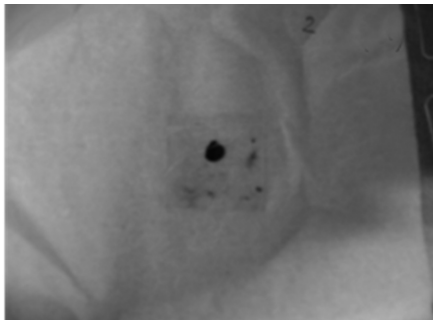

Figure 7 (c)

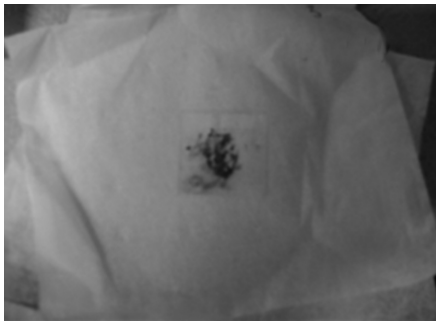

Figure 7: (a) Shown is a functionalized slide that was pre-rinsed in methanol and acetone, plasma cleaned for 12 minutes, and received a reduced concentration of palladium solution. Metallization did not confirm the predetermined pattern; metallization appeared on the sides and the most of the metal observed was not concentrated in the middle of the slide. Most of the copper deposit came off during the rinsing. (b) A functionalized slide that was pre-rinsed in methanol and acetone, plasma cleaned for 12 minutes, and received a reduced copper solution. The best results were achieved during this study. The substrate's exposure to the copper solution was limited to 2 minute and 24 seconds. None of the metal deposit peeled off during the rinsing. (c) This non-coated slide was pre-rinsed in methanol and acetone, plasma cleaned for 12 minutes, and received a reduced concentration of palladium solution. Metallization occurred over the entire slide. The stamp used for this slide did not form a good seal to the glass.

Conclusions and Recommendations

The results confirm conclusively that amine groups could be selectively visualized using the selective metallization technique. It was also concluded that technique was inadequate to improve microarray technology. However, it is believed that with the right parameters, the technique could become more selective visualization tool that could offer a cost efficient and simplistic way to observe a substrate that has been patterned with micro sized amine areas. By manipulating the parameters of the study, specific variables in the experiment could be observed and evaluated to confirm if those variables led to a more selective metallization technique. Some of the parameters and variables tested were the plasma cleaning time, the use of the clamp to keep the PDMS stamp in place, placing fluorinated silanes on the substrate, the time the copper solution was left on the substrate, palladium solution concentration, the ratio of formaldehyde to copper solution, functionalizing the stamp, pre-rinse in acetone and methanol, and the use of HF to pattern the substrate.

Testing each variable offered a different perspective and sometimes led to other possible variables that could be tested. The most critical variables found were the length of the plasma cleaning time, reducing of concentration of the palladium solution, and the amount of time the copper solution was exposed to the substrate surface. Reducing the concentration of the palladium solution and limiting the metallization time offered the best results observed for the selective metallization procedure (Figure 7(b)). Both variables influenced the rate at which the copper solution was plated on the substrate and amine pattern.

Lowering the concentration of the palladium solution causes a reduction in the palladium seeds on the surface of the substrate, with more of the palladium seeds migrating to the area containing the amines. This allows a greater rate for metallization to occur on the amine groups and not on the surface for the slide. Since more palladium seeds are attracted to the amine groups than the surface of the glass, then the amine-functionalized region of the slide (the area where the protection stamp was placed) should react quicker with the copper solution thus the metallizing faster than any contaminants on the glass surface or the glass surface itself. It was observed that as the copper solution was exposed to the surface of the substrate for prolonged lengths of time a slower rate of metallization took place on the amine functionalized region and more metallization began to occur on other areas of the substrate. The continued metallization of the protected areas of the slide led to unwanted build up of copper that had a tendency to peel off the substrate during the water rinse. This skewed the results of the slide by giving the appearance that the protected area did not receive the desired metal deposit. By changing the concentration of the palladium solution and the exposure time of the copper solution, a greater difference in the time between the substrate and amine group metallization was achieved. Moreover, by controlling the exposure time more accurate results were captured to exemplify the selective of the metallization process.

Extending the length of the plasma clean from 3-15 minutes and pre-rinsing the slide prior to the plasma procedure were successful ways to create a more selective metallization process by eliminating more surface contaminants. These variables were also used in producing the best observed data (Figure 7(b)). The cleaning techniques that were used on the non-coated slides (e.g. increasing the cleaning time to about 6 minutes), reduced the amount of metallization to virtually none. However, for the functionalized slides substantially greater plasma cleaning time was needed to achieve the same results as the non-coated slides; usually about double the cleaning time used for non-coated slides. By adding a clamp to secure the PDMS stamp, the cleaning time had to be increased further and the same quality of results were not received using the functionalized substrate. Nevertheless, the time increase and stamp did work for the non-functionalized glass. The clamp was initially added because a more defined square shaped protective area was observed in the metallization results when the clamp was used; however, in the long run it was proven to be ineffective to achieve a more selective technique.

While lengthening the plasma cleaning time produced better results on slides, this cleaning also generated some unexpected results, such as the PDMS losing its adhesive ability and hydrophobic interactions on the substrates. The observed hydrophobic traits took place in the area protected by the PDMS stamp. In that area, the contact angle of the copper solution on the substrate changed, and the copper solution and water (during the water rinse before metallization) would flow more readily to the unprotected areas rather than staying in the center of the slide (the area desired for metallization). One plausible explanation for the interactions suggest that as the plasma clean oxidized the exposed areas of the glass the protected area of the glass remains untouched. This caused the exposed glass areas to be significantly more polar than the area of the glass under the stamp (Birch, 2003).

After being repeatedly used for various experimentation, the PDMS stamps used began to lose their adhesive ability. This skewed metallization results, particularly for one experiment (Figure 7(c)). The reduced adhesive ability did not allow the stamp to form an air tight seal on the surface of the glass, and this probably allowed the oxygen plasma to clean portions of amines from the protected area. It was concluded that the reduced effectiveness of the PDMS piece was due to an over-exposure of plasma cleaning which causes the chemical makeup of the protection stamp to change. Instead of the silanol molecules of the PDMS protruding from the surface of the stamp to form bonds with the glass, these groups became attached to the oxygen bound of other silanol groups on the surface of the PDMS stamp (Katzchenburg, 2005).

The variables that appeared ineffective were the ratio of formaldehyde to copper solution, functionalizing the PDMS piece, the use fluorinated silanes and the use of HF. However, that classification does not mean that the variables could never be useful in future experiments of selective metallization. While HF is an extremely effective etching agent, its use in this study produced no usable results. For both slides dipped in HF, metallization still occurred on the entire slide. The procedure followed was to immerse the slide for a few seconds, to prevent the acid

from changing the chemical nature of the PDMS stamp while allowing the HF time to eat away exposed glass.

While the fluorinated silane did not improve the metallization technique, it was a great diagnostic tool. The reasoning behind the use of the vapor coating of fluorinated silanes was to determine if hydroxyl groups were reacting with the palladium solution to produce metallization. The fluorinated silane reacted with the hydroxyl groups preventing them from reacting with the metallization. However, metallization still occurred in unprotected areas, suggesting not all amine groups were removed from unprotected areas of the glass.

By functionalizing a PDMS stamp, the goal was to stamp amine groups on the surface. However, for this experiment most of the slide metallized which suggests that amine groups began to spread all over the slide from the area where the stamp was initially placed. The strategy behind the increase of 1:5 to 1:1 of formaldehyde to copper solution was to reduce some of the hydrophobic interactions that were observed after the lengthy plasma cleaning. By placing equal amounts of non-polar formaldehyde and the polar copper solution, the hydrophobic interactions would not be observed. The result was that no hydrophobic interactions were seen and neither was any metallization. By increasing the formaldehyde in the solution the metallization time was slowed. This explanation was made after no metal deposits were observed in the copper and formaldehyde mixture even though the mixture was left standing for an excess of twenty minutes.

Recommendations

One of the parameters which could potentially improve the metallization technique is a reduction in the amount of amine functionalization. This approach can be applied by using a brush to coat the surface of the substrate, vapor deposition instead of submerging slide in an aqueous amine solution, or by placing a mask on the cover slip that leaves a space where amine functionalization is wanted. By reducing the amount of amine groups coming in contact with surface of the glass less amounts of amine groups will have to be cleaned or etch off the glass surface during the patterning. For instance, with the use of the mask, the step to remove amines would be eliminated all together.

HF is an effective cleaning procedure for the metallization process, so using HF etching to pattern amines may still be used, however longer immersion time in HF maybe required. According to Blalock (2003), "5% or less HF used at room temperature [normally requires an etching time] for [at least] 10 minutes" (p 5). HF can also be used to supplement, the plasma clean after the plasma clean has etched away most of the amine groups.

The vapor deposition of fluorinated silanes on the slides still is an important diagnostic tool and a viable option to improve the technique. The silane could be selectively placed on the surface of non-functionalized glass in exposed areas after sufficient plasma cleaning; then the slide could be functionalized and finally metalized.

The use of a clamp to hold the PDMS was proven ineffective, yet the technique of making sure the PDMS stamp does not move during the plasma clean is still a logical argument. A technique to secure the PDMS stamp more securely to the glass slide so that the PDMS piece makes more contact with the slide can be devised based on methodologies proposed in "E-polymers" by Frank Katzchenburg. The technique would create the effect of using the clamp without inhibiting the efficiency of the plasma clean.

Through the creation of a more selective metallization technique for micron size amine areas on glass, new techniques for the creation of nano-microarrays or nanoarrays can be realized. This would allow microarray technology to expand, causing a revolution in detection systems, biotechnology, food and agricultural quality, health fields, RNA technology, genomics, and proteomics (Blalock, 2003; Schena, 2003; Schena, 2005). The overarching effects of this occurrence would be an increase in the living and health standards, which would impact every person in the world.

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