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How to Use this Manual

When you read the title of this module, you may realize that it is not so broad as “chemistry” or even subdivisions such as “organic chemistry” or “analytical chemistry”. This module focuses on a very specific area of research that is prominent in the scientific community. If you have trouble getting an idea of what the module is about or what you will be doing from the title, do not panic! As you work through the module, you will come to understand the details and become a researcher in this exciting area.

As you may notice when looking through this manual, there is an introduction section followed by several weeks of experiments. Be sure to read the introduction before you begin attempting the experiments – it provides you with the necessary background information to understand the big picture of what is happening in the laboratory.

After the introduction, the module is divided into sections by laboratory period. Each laboratory period section includes an introduction of its own and an overview. These introductions, unlike the introduction to the module, provide any background knowledge necessary to work through that particular week’s in-lab work and analysis. The overview is designed to help you keep the rest of the module in mind and make connections between the different laboratory periods.

Following the overview are pre-laboratory exercises. You should complete these exercises before attempting any of the procedures. After these exercises are a materials list and the procedure, which provide information on what you will be doing in the laboratory. The post-laboratory exercises following the procedures require that you reflect on your laboratory experience, answer theoretical questions, and analyze the data you obtained in the laboratory.

As you read through the module, you may notice that some words are bolded and have footnotes. The definitions of the bold words can be found both in the footnotes and the glossary in the back of the module.

It is important to keep in mind that this is a research module and not simply a set of experiments for you to perform. Your focus should be more on how you go about answering and developing your research question and having a conclusion based on results, rather than a specific answer. Remember that in research, results do not always come easily or as expected; it is the process that will help you develop into a scientist.
About the Author:
Dr. Albena Ivanisevic is an assistant professor in the Weldon School of Biomedical Engineering and the Department of Chemistry at Purdue University.

Her research is focused on the fabrication and evaluation of biological and chemical architectures. Most of her work is centered around chemically modifying surfaces in different ways which can allow them to be used as sensors. The surfaces she investigates have the potential to be used as novel biomaterials, sensors and optoelectronic platforms.

Recent research in the Ivanisevic Research Group involves developing templates for positioning of nanoscale molecular wires onto surfaces and, also, developing techniques for the engineering of an artificial retina.

From this module, Dr. Ivanisevic hopes to gain information on different protecting groups and localized deprotection methods. With this data, the Ivanisevic Research Group hopes to develop an efficient method of protecting and deprotecting surfaces. This method will ultimately be used to develop a biosensor.

Recent Publications:


Group Home Page:
http://bmew.ecn.purdue.edu/Ivanisevic/
I. Introduction

1. The importance of chemical surface detectors
As you read the title of this module you may be wondering about several things: What are biochips? What is a ligand? Why are ligands attached to surfaces? What are biosensors? Will I actually make a biosensor? What are biosensors used for? Will I have the need to use a biosensor in my everyday activities? If you do not know the answers to these questions, do not panic! You will discover all of these answers on your own in the next 6 to 8 weeks. I hope you have fun learning and exploring this new area!

Biosensors can be used to identify and quantify a variety of different biological molecules. For example, home pregnancy tests detect human chorionic gonadotropin (hCG) in the urine of a pregnant woman. DNA evidence can be detected and specifically identified. Diabetics use biosensors to measure their blood glucose concentration. Although these uses for biosensors have been established, there are many possible uses for biosensors that have yet to be examined. There is also a need to research and improve the design and manufacture of biosensors.

2. The anatomy of a sensor

For the purpose of this module, the anatomy of a sensor consists of three key components: the surface, the self-assembled monolayer (SAM), and the protection region. The surface can be composed of a variety of substances such as glass or gold. The SAM consists of a single layer of molecules attached to a surface. The protection region consists of a chemical that protects areas of the exposed SAM from being chemically altered.

In order for the SAM to attach to the surface, cross-linking must occur. Cross-linking is the process of covalently bonding two molecules that are un-reactive toward each other by a chemical group that is reactive to both. Cross-linking reagents contain ends that...
react with specific functional groups. Therefore, it is important to choose a surface and a cross-linking reagent that react strongly with one another. In this module, you will be preparing a SAM that has been previously attached to the cross-linking reagent, but not the surface.

![Figure 2 - A schematic representation of cross-linking.](image)

### 3. Protection and deprotection
Protection chemistry is a common method of organic synthesis that prevents the reaction of a specific chemical group from occurring. In solution-based chemistry, this method is used to leave a functional group unaffected, though some other part of the molecule of interest will remain reactive. The method is similar to the analogy of a laboratory glove: without it, your hand could be hurt by a number of substances in the lab. The glove, on the other hand, is fitted to your hand and protects it from harm. An example of this is having two functional groups: A and B. These can be on the same compound or be parts of two separate molecules in a solution. If you only want to react functional group A, you will have to protect functional group B with protection chemistry that won’t affect group A. The protecting group used would be fitted specifically for that particular functional group.

![Figure 3 - a) A reaction in which a reactant reacts with two functional groups. b) To protect the circular functional group from reacting, a protecting group is used that is specific for that functional group. c) The original reaction, a, is then run following the addition of the protecting group to allow only the desired functional group to react.](image)
Deprotection chemistry is the method by which protecting groups are removed without altering the rest of the molecule. This can be done with the use of acid/base solutions, halogens, reduction/oxidation reactions, electrochemical or light-based methods, or heat. Using the example from above, once functional group A is reacted, the protecting group of functional group B is removed. Research to examine highly selective methods to perform protection chemistry on surfaces has been observed in scientific literature. However, in this module you will adapt what has been achieved to use micro-contact printing in the deprotection process.

![Figure 4 - The deprotection of the product from reaction c in figure 3. Note the matching shades and complementarity of the deprotecting agent and protecting group. This represents the strong affinity and specificity that the deprotecting agent has for the protecting group.](image)

4. Microcontact Printing

Soft lithography describes techniques for fabricating or replicating structures on a micro- or nanometer scale. This can be done using elastomeric materials or other systems of depositing molecules to construct these features. The key feature of these methods is that they do not remove material from the substrate to create patterns, as in e-beam lithography. Rather, the techniques use more gentle means to create patterns given the properties of the surface.

![Figure 5 - The chemical formula for polydimethylsiloxane (PDMS).](image)

Micro-contact printing (μ-contact) is a soft-lithographic technique that uses a patterned elastomeric stamp formed from polydimethylsiloxane (PDMS). The stamp deposits layers of a given material on a surface through conformal contact. Conformal contact

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1 Elastomeric: An adjective describing a substance as an elastic substance occurring naturally or produced synthetically.
means that the PDMS stamp adapts **elastically**\(^2\) to the surface without leaving small, unintentional gaps in the protection pattern. Layers of material are deposited because the stamp is “**inked**\(^3\)” with a desired solution that will coat the stamp and form a **solid solution**\(^4\). The stamp is then dried and pressed onto the surface to deposit material from the solid solution.

![Image](image.png)

**Figure 6** – This is an example of a product done by μ-contact printing. In this module, you will be using μ-contact printing in a different way. Scale-bar in bottom-right corner is 200μm. From L. Lauer, S. Ingebrandt, K. Scholl and A. Offenhauer, *IEEE, Trans. Biomed. Eng.* 2001, 48, 838-842.

5. **Applications**

Much is known about biosensors and how they can be used in real world environments to gain information and solve problems. A few examples were given at the beginning of this introduction section and many more could easily be listed. For example: ecological uses for detecting air and water pollutants or monitoring for food safety.

The data that you gather regarding protection chemistry and μ-contact printing will be used by the Ivanisevic lab group to develop biosensors that can be used for the detection of ionic species.

6. **What is not known**

In the previous sections, information about chemical surface sensors and how they work was provided. Although much research has been conducted, there is still much to learn. For example:

- Whether or not a number of prospective protection chemistries are applicable to μ-contact printing.
- Which of the above said methods are efficient or effective for the desired end?

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\(^2\) Elastic: An adjective describing a substance being capable of returning to its original length, shape, etc. after being stretched, deformed, compressed, or expanded.

\(^3\) Inked: A chemical solution applied to the stamp that is used for protection or deprotection.

\(^4\) Solid solution: A solid-state solution made of one or more solutes in a solvent. The crystal structure of the solvent remains unchanged by the addition of solutes, or the solute being incorporated into the solvent crystal lattice substitutionally.
7. Sample Hypotheses

In the last few weeks of this module, you will be designing and carrying out your own research experiment within the parameters discussed above. You will use some or all of the techniques you learned in the first three laboratory periods of the module to answer your research question.

The experiment that you design will be based on a hypothesis that is related to the development of surfaces that can be manipulated using $\mu$-contact printing. At that time, we will provide you with some additional guidance for developing a useful and testable hypothesis. Here we will simply provide some examples to give you an idea of what questions still need to be answered in the development process.

Sample hypotheses:

- Changing the ratio of silanes will change the ability of the surface to be modified with a fluorescent group.
- The pH of the buffer used during the fluorescent tagging of a surface will change the amount of fluorescence.
- Other fluorescent tags can be attached to a surface that can be labeled with dansyl chloride.
- Dansyl chloride does attach to some surfaces but does not fluoresce. This may be because of several factors, which can be tested by altering the surface modification.

8. Module Calendar

This module will take place over 6 to 8 laboratory periods (depending on the exact schedule defined by your own instructor.) In the first three laboratory periods, you will learn three specific techniques:

1. Modifying glass surfaces with alkoxysilane reagents.
2. Measuring the properties of surfaces with contact angle measurements.

You will then use all or some of these techniques to test the experimental hypothesis you develop. This outline is summarized in the module calendar table given here.

<table>
<thead>
<tr>
<th>Lab Session</th>
<th>Activity</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Contact angle measurements</td>
<td>Learn how to detect whether or not a surface has been altered and the principles of hydrophobicity and hydrophilicity and contact angles.</td>
</tr>
<tr>
<td>2</td>
<td>Stamp making and designing</td>
<td>Learn how to specifically manipulate certain regions of a SAM.</td>
</tr>
<tr>
<td>3</td>
<td>Protection and deprotection</td>
<td>Modify a surface using a fluorescent label that reacts with groups on the surface.</td>
</tr>
<tr>
<td>4-6</td>
<td>Independent research</td>
<td>Develop problem solving techniques, design research project, apply acquired knowledge, and answer research question.</td>
</tr>
</tbody>
</table>
9. What is the big picture?
During this module you will be learning a number of laboratory techniques. More importantly, however, you will be doing real science. You will be testing a novel hypothesis and your results will contribute to scholarly research. Specifically, you will help the Ivanisevic research lab develop an ion sensor that can be used in numerous applications. Keep in mind that, at the end of the module, you will be sharing your data and results with the Ivanisevic research group via an online form. Your data will become part of their research database.

The overall process of carrying out research is an important experience for anyone studying science. You will find that there are topics throughout this module that relate directly to the lessons you have learned in your chemistry lecture courses, such as chemical structures, hydrophobicity and hydrophilicity, and functional groups. In the end, this is a novel opportunity for you to receive an educational experience to aid in your melding of skills in hypothesis development and critical thinking, as well as applying concepts you’ve learned.

II. Laboratory Period 1 – Contact Angle Measurements

1. Introduction
We are surrounded by a multitude of surfaces. For instance, you might think of glass surfaces as smooth and of sand surfaces as rough, or of a wood panel as having a specified height and width. In this module, you will learn how to characterize the chemical properties of a surface. More specifically, you will be looking at ways to examine the chemical surface composition and exercise your ability to change the composition and properties of the surface. You will learn to classify surfaces based on their dimensions, their interaction with water, and their surface structure. Through chemical manipulations you will also learn how to modify the surface in ways that allow sensors to be designed on glass.

An easy way to get a sense of some of the properties of a surface is to examine how liquid beads when dropped on it, if a water drop beads then it is considered non-wetting, and if a water drop spreads out and is considered wetting. An example of a “non-wetting” surface is a freshly waxed car. When water contacts this surface it beads up rather than spreading out. As the wax is degraded, the surface of the car becomes more “wetting” and the water is able to spread. Assessing the surface’s resistance to wet or not to wet by a given liquid gives us an idea of the surface tension. Surface tension is the force at the interface of liquid and gas that is responsible for forming a barrier and holding a droplet together. The tension always exerts pressure that is tangent along the surface.

It is very useful to design experiments to determine what it takes to separate a solid from a liquid interface. In order to accomplish this separation, one needs to do reversible work.
Reversible work can be defined as the difference in free energy ($\gamma$) between 2 states, the liquid and the solid. In an effort to determine surface free energies, one measures contact angles. The surface free energy values allow one to understand the wetting and adhesion properties of surface materials. Understanding how contact angles are utilized in experiments to determine surface properties is very important for the design and evaluation of surfaces that can be used in biosensors. The next section in this module presents a detailed description of contact angle measurements.

There are a variety of organic and inorganic surfaces that have the potential to serve as a foundation for biosensors. One might choose such surfaces because of their mechanical properties. For instance, surfaces that are hard to break will be preferred over ones that are easy to break in biosensor applications where conditions such as pressure and high forces are expected. A different criterion for choosing a surface might be its optical properties. For example, surfaces that are transparent can be used in certain spectroscopic methods. Electrically based biosensors will benefit from surfaces that are conductive. Metal surfaces can be used for such applications.

Based on the few examples given so far, you can see that a wide range of organic and inorganic surfaces are possible for the fabrication of a biosensor. It is not always feasible to use a material in its natural state for surfaces in biosensor applications. One often needs to chemically modify surfaces in order to produce the right binding environment for biological analytes. In this activity you will begin to learn how to carry out such chemical modification of surfaces.

2. Contact angle measurements

The contact angle, $\theta$, is a measure of the wetting of a solid by a liquid. It is defined as the angle formed between a surface and the tangent at the point of contact of a liquid droplet (Figure 4). A droplet of liquid forms when three forces of interfacial tensions are in equilibrium: 1) the solid and liquid, 2) the liquid and gas and 3) the solid and gas.

A contact angle goniometer can be used to quantify the wettability of a surface with a given liquid. When a surface interaction is wettable, causing the liquid to disperse, the molecules in the liquid have a stronger attraction to the solid surface than to each other. When a surface interaction is non-wetting, causing the liquid to bead, the molecules in the liquid are more attracted to each other than to the solid surface. Surface interactions with a contact angle less than 90° are referred to as ‘wetting’; while a contact angle greater than 90° is referred to as wetting. A completely wettable surface would have a contact angle of 0°. Surfaces such as glass and metal tend to yield low contact angles.
with water as a liquid, while surfaces such as plastics produce high contact angles. For example, the contact angle of water on clean glass is approximately $14^\circ$ while the contact angle of water on paraffin wax is $109^\circ$.

![Figure 8 - a) A contact angle less than 90°, representing a wetting surface interaction. b) A contact angle greater than 90°, representing a non-wetting surface interaction.](image)

Contact angle measurements can be used to evaluate the hydrophilicity/phobicity of a surface, or to monitor the coating of a surface. Hydrophilic surfaces have smaller contact angles compared to hydrophobic surfaces when measured using water as the liquid. Chemical groups attached to a surface can alter the hydrophilicity/phobicity of the surface. A hydrophilic chemical group (relative to the native surface) will decrease the contact angle; a hydrophobic chemical group (relative to the native surface) will increase the contact angle. An example is oil, a non-polar liquid, on glass, a polar surface.

Although in this activity you will be measuring equilibrium/static contact angles, advancing and receding contact angles can also be measured in future activities. An advancing contact angle is measured while the drop of liquid is expanding; the receding contact angle is measured as the drop is contracting. The advancing contact angle is normally greater than the receding angle. This can be due to a contaminated surface, surface roughness, or reconstruction of the surface by the liquid. A small difference between receding and advancing contact angles ($<5^\circ$) signifies that the surface is relatively free of contamination, smooth, and well organized.

3. Chemical modifications using silanes.
A key substance for modifying a glass surface is a class of compounds called silanes. Silanes are compounds with a central silicon atom bonded with four single bonds to other atoms or groups. One kind of silane has three “X” groups that are easily replaced by water or another substance that has an OH group (like the surface of glass). Because of the different “X” groups possible, silanes are versatile compounds that allow one to change the properties of oxide-terminated surfaces efficiently. Examples of such oxide-terminated surfaces include quartz (SiO$_2$) and TiO$_2$. TiO$_2$ is a material that is commonly used for a variety of biomedical implants such as hip replacement joints.

Silanes have four distinct parts: the hydrolysable groups, the organofunctional groups, a linker part, and an end functional group. The general formula of such a silane is “R-(CH$_2$)$_n$-SiX$_3$.” The hydrolysable groups are used to anchor the molecule to a surface. The organofunctional group allows flexibility in terms of the chemical functionality is attached to the top of the surface. The linker part is used to “dial-in” an appropriate distance between the surface and the end functional group specified by R. The end functional group is the end of the linker chain and is what reacts when the silane is attached to a surface. An example of such a silane is trimethoxypropylsilane,
CH$_3$CH$_2$CH$_2$Si(OCH$_3$)$_3$, which has three methoxy groups as the “X” groups and a three carbon propyl chain as the fourth group.

Figure 9 - A silane coupling agent. The R group is the organofunctional group, the carbon chain (n) is the linker, and the X groups are hydrolysable.

Through the hydrolysable groups, silanes can be attached to the surface either by hydrogen bonding or by covalent bonding. For a number of applications a covalent attachment, which is stronger and more stable, is preferred. Silanes require four steps in order to form a covalent bond to the surface. In the first step, a hydrolysis of the three labile groups, X$_3$, takes place. In the second step, a condensation reaction is carried out. In the third step, the transformed silane binds to the surface using hydrogen bonds. The fourth and final step is referred to as curing or drying because it is carried out at elevated temperatures and results in a covalent bond between the surface and the silane molecule. This final step creates crosslinking in the silane which effects the wettability of the surface. Following are examples of the four steps the silane goes through to attach to a surface.
In this module we will use two different types of functional groups. The first is a primary amine (-NH₂) and the second is a methyl ester (-COOMe). These two different classes of silanes provide for different wettability of glass surfaces.

4. **Overview of this laboratory activity**

In this laboratory activity, you will learn how to measure contact angles on surfaces. At the beginning, you will be introduced to the contact angle measurement apparatus, a goniometer. Subsequently, you will have the opportunity to see how the surface properties of glass change as a result of handling the slides. Later in the research module, you will be chemically modifying surfaces. Contact angle measurements can be used to determine whether or not a surface has truly been chemically modified in a given reaction.
5. The contact angle goniometer

A goniometer is an instrument used for measuring geometric angles. Here we will be describing two different measurement apparatuses. One measurement apparatus is commercially made, while the other is considered homemade. Regardless of which goniometer is used, the primary components are the same. The primary components of a contact angle goniometer are: a light source, a movable stage, a lens to focus light, and a measuring screen. The general idea is that the light moves over the slide holding a liquid drop, and the image is then magnified with a lens. The silhouette is portrayed on the measuring screen and the contact angle can be determined from this image. The role of the stage is to position the sample slide and the droplet in an appropriate location in front of the lens to allow a focused image onto the measuring screen.

Figure 11 - The Tantec goniometer.
Consult with your instructor about the instrument you will be using, and if your instrument is significantly different from either described in this section; be sure to address this in your lab notebook. Write a detailed description of how your instrument functions as well as a protocol for collecting data on your slides.

6. Measuring contact angles
This procedure is generalized so that it can be used with a variety of apparatuses, including the two described in the previous section. Begin by placing the sample slide on the stage with the appropriate side facing up. It is important to use care when handling the slides. **DO NOT TOUCH THE SLIDES WITH YOUR BARE HANDS UNLESS INSTRUCTED TO DO SO.** Always use forceps or wear gloves when moving glass slides. When the slide is on the stage, position it so that it is near the lens. If you are going to move the slides by hand, be sure to only touch the edges of the slide.

Turn on the light source and readjust the position of the sample slide on the movable stage so that the image portrayed on the measuring screen is sharp and aligned with whatever sort of measuring screen being used. When the image of the slide is focused, drop a single drop onto the slide. Be sure that the droplet is set near the edge of the slide nearest the lens. **Adjust the stage again so that the side of the droplet is focused and positioned with one edge of the droplet along the bold vertical line of the measuring screen and the bottom of the water droplet is along the bold horizontal line as seen below.**
Figure 13 - An image of a water droplet on a slide that is projected onto a measuring screen. Note that the edge of the droplet is lined up with the vertical line and the bottom of the droplet is lined up with the horizontal line. The red line passing through the image is referred to as a “hairline”.

Figure 14 - A contact angle measuring screen of a homemade apparatus. Note that the image of the water droplet is inverted.

To collect the data, you can either use a rotatable hairline affixed to the measuring screen as seen in Figure 7, or can simply draw on a paper measuring screen as seen in Figure 8. If you are working with a homemade goniometer and are using chart paper as a measuring screen, it may be easiest to simply trace the image of the droplet and then remove the sheet of paper from the apparatus. The next step is to determine the half angle, which is simply one half of the contact angle. This can be done by rotating the hairline, or drawing a straight line that begins in the center of the chart paper and passes through the top of the droplet image at the peak of the droplet. The peak of the droplet should lie halfway from one edge of the droplet to the other. Determine the angle of the
straight line from the horizontal. This is the half angle ($\theta_{1/2}$). Now multiply the value of the half angle by two and viola!, you have determined your contact angle.

Figure 15 - Illustration of contact angle determination on a measuring screen.

7. **Pre-lab requirements**
Write the introduction and experimental sections for this laboratory in your notebook. Use your own words to describe the purpose and overview of today’s activities. Your experimental section should include detailed instructions that will allow you to carry out the experiment without needing to refer to this manual. Also formulate some ideas about how to address the contact lens experiment in Part 2.

8. **Materials**

- Pre-cleaned glass slides
- Forceps
- Fine Point Permanent Marker to label slides
- Aminopropyltrimethoxysilane – APTMS
- Propyltrimethoxysilane -- PTMS
- Staining jars
- 1 mL Mohr pipettes
- Graduated cylinder
- Graph Paper
- Protractor
- Goniometer

Note: if you do not have a commercially available contact angle meter, you may construct one using the following reference: M. Dionisio, J. Sotomayor “A Surface Chemistry Experiment Using an Inexpensive Contact Angle Goniometer,” J. Chem. Ed., v. 77 (1), pp. 59-65. Ask your instructor which version of the instrument you will be using and thoroughly read through the description. It may be helpful to compare and contrast the differences between the instrument you will use and the one that is described in Part 6.
9. Procedure

Contact Angles on Glass slides

The slides that are used in this section have been pre-cleaned by the manufacturer, or by the instructor, and are ready to be used. IT IS VERY IMPORTANT THAT THE SURFACES DO NOT GET CONTAMINATED FROM HANDS OR FROM PARTICLES ON TABLETOPS. Always use forceps or hold the slides by the edges with gloves on when moving them.

First, you will collect contact angle data on pre-cleaned glass microscope slides. Give the slide a quick inspection to make sure that there are not any large visible contaminants on the surface. If the slide appears to have dust on it you may rinse it with ethanol then dry with a kim-wipe, or lens cloth, or else choose another slide from the box. Collect contact angle measurements at three distinct points on the slide following the procedure outlined in Section 6 of this laboratory period. After you have completed the measurements, remove the slide from the movable stage and place it onto a labeled paper towel on the lab bench. Repeat this procedure for three pre-cleaned glass slides.

The next sample that you will evaluate is a reference for you to compare to your cleaned slide. To prepare this slide remove it from the box and handle the surface with your bare hands. As you handle the surface you should see your fingerprint being transferred onto the glass. Continue to touch the slide until the entire surface has been coated with fingerprints. After you have finished contaminating the slide, collect contact angle measurements at three spots on the slide. Remove the slide from the stage and place it onto a separate labeled paper towel for later. Repeat this procedure for three dirty glass slides. Please share glass slides among the class, so exchange dirty glass slides with different groups until you have tested three different dirty slides.

Surface modification of glass slides with amino-silane.

Make up a 2% (v/v) aqueous solution of aminopropyltrimethoxysilane (APTMS) in a polypropylene staining jar by mixing 800 μL of APTMS with 40 mL of distilled water. Mix the solution by screwing the lid onto the jar and gently inverting a few times. Using forceps carefully place 5 pre-cleaned glass slides into the jar of solution. Ensure that most of the slide is submerged. Leave the slides in the solution for 10 minutes. Very carefully pour out the APTMS solution from the staining jar into an appropriate waste container. Do not remove the slides from the container, only the solution. Rinse the slides 3 times with distilled water in the staining jar, discarding the solution into an appropriate waste container each time.

After the slides are rinsed remove them from the staining jar and place them into a drying oven at 120°C for 10 minutes to remove water and crosslink the silane. Make sure to remember which end was least submerged. Carefully remove from the oven using a hot mitt and forceps – the glass will be hot! After the slides are removed from the oven place them onto a surface where they can cool to room temperature. Be sure to label your slides. If the slides are laid onto a surface with one side up be sure to either mark the side that is up or pay careful attention so that the same side always remains up. After the slides are cooled, take one slide and measure the contact angle using distilled water.
Repeat the measurement 3 times per slide. Be sure that you collected the data from the side that was facing upwards.

Make sure to safely store your slides from this week for use next week. Proper storage includes having a way to keep the surfaces uncontaminated while they are in your drawers. Be careful about stacking slides because you can contaminate the surfaces easily that way.

**Surface modification of a glass slide with propyltrimethoxysilane**

Make up a 2% (v/v) solution of propyltrimethoxysilane (PTMS) in a polypropylene staining jar by mixing 800 μL of PTMS with 40 mL distilled water. Mix the solution by screwing the lid onto the jar and gently inverting a few times. Using forceps carefully place 5 pre-cleaned glass slides into the solution. Leave the slides in the solution for 10 minutes. Very carefully pour the solution from the staining jar into the appropriate waste container. Do not remove the slides from the container only the solution. Rinse the slides 3 times with distilled water in the staining jar, discarding the solution into an appropriate waste container after each rinse.

After the slides are rinsed remove them from the staining jar and place them into a drying oven at 120°C for 10 minutes to remove water and crosslink the silane. Carefully remove from the oven using a hot mitt and forceps – the glass will be hot! After the slides are removed from the oven place them onto a surface where they can cool to room temperature. Be sure to label your slides so you know what they are. If the slides are laid onto a surface with one side up be sure to either mark the side that is up or pay careful attention. Repeat the contact angle measurement procedure from the APTES slides.

Make sure to safely store your slides from this week for use next week. Proper storage includes having a way to keep the surfaces uncontaminated while they are in your drawers. Be careful about stacking slides because you can contaminate the surfaces easily that way.

**10. Post-laboratory analysis and results**

Most, if not all, of these calculations can be done with spreadsheet software. If this is your first exposure to these equations you are encouraged to work out the problems by hand, but in your future as a scientist knowing how to calculate these values on a computer will simplify your data analysis.

a. Calculate the average and standard deviation of each data set you collected in the lab. Do you think the data you collected is accurate? Do you think the data you collected is precise?

b. Do the contact angles measured on clean vs. unclean glass surfaces make sense? Why and why not?

c. What was the contact angle after coating your glass slides with the amino silane? Does the change in contact angle make sense when compared to clean glass? Why or
why not? Is there a statistically significant difference in contact angle between the amino silane coated surface and clean glass?

d. What is the structural difference between the non-hydrolysable groups on propyltrimethoxysilane and aminopropyltrimethoxysilane? How can this account for differences in the contact angle on the slides modified with the different silanes?

e. What was the contact angle after coating your glass with the propyltrimethoxysilane? Does the change in contact angle make sense when compared to clean glass? Why or why not? Is there a statistically significant difference in contact angle between the propyltrimethoxysilane coated surface and clean glass? What chemical phenomenon accounts for this change?

III. Laboratory Period 2 – Stamping on Solid Surfaces

1. Introduction
Sensors can be developed in a number of different methods. The method we will use in this module is soft lithography. Soft lithography is an approach for micro- and nanofabrication of structures on a surface. It is a convenient, effective, and low-cost method for the production of micro- and nanostructures. The fundamental part of soft lithography is an elastomer that has patterned structures on its surface. The use of an elastomer is important because it has conformal contact with surfaces over large areas and is easy to replicate. In this module, we will be using a polydimethylsiloxane (PDMS) elastomer for microcontact printing to alter a protecting group bonded to a self-assembled monolayer.

2. PDMS stamping
Polydimethylsiloxane (PDMS) is a common silicon-based organic polymer that is widely used and is known for its many properties. It is transparent, non-toxic, non-flammable, flexible, highly compressible, relatively inert, and can be used at a wide range of temperatures. The chemical formula for PDMS is \((H_3C)[SiO(CH_3)2]_nSi(CH_3)_3\) and is made up of a variable number of repeating \(SiO(CH_3)_2\) monomer units.

\[
n \ [(CH_3)_2SiCl_2 + H_2O] \rightarrow n \ [SiO(CH_3)_2] + 2n \ HCl \rightarrow (H_3C)[SiO(CH_3)_2]_nSi(CH_3)_3
\]

Figure 16 - The formation of polydimethylsiloxane from dimethylchlorosilane and water.

In order for the PDMS polymer to form, the \(SiO(CH_3)_2\) monomer must be formed from dimethylchlorosilane and then polymerized to make the polymer. Polymerization is a reaction in which two or more monomers are bonded together through different reaction mechanisms to form longer chains. Polymers are essential because they have the ability to take on any desired shape. Polymers are generally formed as liquids and take the shape of their container as they solidify.

3. Overview of this activity
In this laboratory activity, you will be use the design for your PDMS stamp that you will design as part of your prelab work. You will then start the protection/deprotection to have slides ready for next week.
4. Pre-lab requirements
Write the introduction and experimental sections for this laboratory in your notebook. Use your own words to describe the purpose and overview of today’s activities. Make sure you have accounted for the time it takes to do certain things and plan accordingly. Your experimental section should include detailed instructions that will allow you to carry out the experiment without needing to refer to this manual. Come up with a feasible design for a stamp pattern that can easily be put on a one inch slide. For a feasible design remember that the slide is only an inch wide. Also make sure that at least half (50%) of the stamp will make contact with the slide when you stamp with it. In Figure 11 there are some possible stamp ideas to get you started.

![Possible stamp ideas](image)

Figure 17 - Examples of possible design ideas. The shaded areas are the areas cut out.

5. Materials
- Sylgard Elastomer 184 kit (Dow)
- Tea candle holders
- Multipurpose transparency films
- Cardboard Pieces
- Fine-tipped permanent marker
- Razor blades
- Glass slides
- Plastic Solo cups
- Foil
- Tongue depressor
- Disposable pipettes
- Glass stirring rod
- Oven able to reach at least 130°C
- Forceps
- DI water
- Soap
- Scissors
- Hole punch
- 1 M NaOH
- 1 M HCl

6. Instructions on how to make a PDMS stamp design
Before micro-contact printing can occur, a PDMS stamp must be made. First, draw the design you came up with in your pre-lab on a transparency film using a fine-tipped permanent marker. Check to see if the entire design fits the bottom of the tea candle holder as well as a majority of it on the slide. The design is then removed by placing the transparency on top of a piece of cardboard and using a razor blade to cut out the design. Once the design is cut out, place it at the bottom of the holder and make sure it is lying completely flat. Figure 12 gives an example of what a cut out design may look like on a transparency.
7. Procedure
7a. Stamp construction
First, make the polydimethylsiloxane (PDMS) stamp based on your design from your pre-lab. The PDMS stamp is made up of a 10:1 ratio of base to curing agent. The balance should be covered with foil when measuring the two components in case of a spill. If you accidentally spill anything on the balances please make sure someone knows because they will need to be cleaned immediately. The 6.0g of base should be measured out in a plastic solo cup. Make sure to mass the solo cup before adding any base to it. After the addition of the base, measure out 0.6g of curing agent into the same cup. Stir this mixture vigorously with a glass stirring rod until only bubbles can be seen in the container. At this point the mixture will be very opaque from all the bubbles in it. Figure 13 gives an example of what constitutes “very opaque.” This insures that the stamp will completely solidify. Then the mixture sits in the solo cup for 30 minutes.
After the 30 minutes, transfer the entire mixture to the tea candle holder containing your stamp design. Make sure to pour in the center of the holder. The mixture will envelop the design transparency film, but it should only be a thin layer. If the transparency film does not stay at the bottom, you can push the film back down with the glass stirring rod. The mixture then sits for another 30 minutes. After sitting for 30 minutes in the tea candle holder there will either be a miniscule amount of bubbles or no bubbles left in the mixture. The mixture is then baked for 30 minutes at 130ºC.

When baking is done, remove the PDMS stamp from the oven and let it cool. Be careful removing your PDMS stamp from the oven – remember that it will be hot! Once cooled, remove the stamp from the holder. Slowly pull the holder away from the stamp, you may cut it if you need to, but be careful not to damage the stamp in any way. After the holder is detached, remove the transparency film. Use a razor to slowly slice out the film without cutting too deep into the stamp. When the film is completely removed, the stamp will then be used for surface application.

![Figure 20 - Picture a is an example of what the stamp will look like when it is removed from the holder. The arrow points to where the transparency film has been enveloped. Picture b is of the stamp after the transparency film is removed from the stamp.](image)

During the waiting periods, you must clean your glass stirring rod and clean up in your lab area. The PDMS polymer is very difficult to remove once it has set, so use acetone on a paper towel to help clean any spills on the lab bench and remove the polymer from your glass stirring rod. The plastic solo cup and the metal tea cup holder need to be thrown away.

7b. Experiments in surface modification

In this part of the procedure, you will make some new APTMS-modified slides and you will study their surface characteristics as you change the nature of the surface group. The APTMS slides are covered with amino groups (NH₂), which are weak bases. Presumably, in water these engage in acid-base equilibria much like ammonia will. However, there is only a single layer of APTMS groups on the glass surface, meaning that there is not enough to affect the overall pH of a solution. On the other hand, the presence of acids or bases in the solution can modify the APTMS groups.
Take two of the APTMS slides you prepared in Week 1. You will treat one slide with a solution of a base (dilute NaOH) and one slide with a solution of an acid (dilute HCl). After treating these slides, measure the contact angle of these two slides.

8. Post-laboratory analysis and results
   a. What factors determine the quality of the printing?
   b. What will happen to the quality of the patterns if your surfaces are very rough or ill defined?
   c. How do you expect acids and bases to change the amine (NH₂) group on an APTES surface? How does this compare to what you observed for the contact angle of the slides you treated with NaOH and with HCl?

IV. Laboratory Period 3 – Stamping of a glass surface with a fluorescent dye.

1. Introduction
   After learning how to study the surface of slides and to prepare a stamp for patterning a modified slide, you are now ready to carry out a test of stamping a slide to obtain a surface that emits a signal. The signal we wish to use is fluorescence, which occurs when a fluorescent dye absorbs light and then emits light of a different wavelength. This may be a familiar phenomenon to you if you have ever seen objects illuminated under "black light," which is a light that gives very short wavelength visible light along with a small amount of UV light. Under these circumstances, certain objects, including notably some clothing and paper, will be seen to glow. That glow is caused by the fluorescence of a dye that has been bound to the clothing or to the paper.

   Fluorescence is one of the most sensitive techniques in analytical chemistry because it allows us to cause a system to emit light. Light is easy to detect, both with our eyes, which are remarkable sensitive especially to the dark, and with instruments. Fluorescence is so sensitive that it can detect when a single layer of molecules is present on a surface, as you will attempt to see in this experiment.

   Most of us are familiar with fluorescence from the effect of “black light” on certain objects, including some clothing. The “black light” appears to be a deep purple but is also emitting some light that we cannot see—right at the upper end of the UV range. This light is absorbed by fluorescent molecules on an object, including fluorescent materials sometimes attached to or absorbed on clothing, and then the object emits a small amount of blue light. We generally cannot see this light in normal conditions, but in the dark it is quite apparent.

   The ability to make an object “glow” through fluorescence is one of the most powerful in analytical chemistry. That is because it is often easy to “tag” an object (whether it is DNA, a protein, or a nanoparticle) with a fluorescent “label.” When we illuminate the object that has the tag, fluorescence appears, often quite brightly, and it makes it easy to
determine that the object is present. Because it is so easy to detect light emission, it is very easy to detect the tag. This can be many millions of times more sensitive than other forms of detection, such as absorption of light (which gives rise to normal color) and electrochemistry.

The fluorescent dye that you will use in this experiment is known as dansyl chloride (Figure 21). Dansyl chloride includes a reactive part and a fluorescent part. The fluorescent part, consisting of a 10-carbon ring system with a dimethylamino \((\text{N(CH}_3\text{)}_2)\) group attached, absorbs long-wave ultraviolet light and re-emits the light in the visible. The reactive part is known as a sulfonyl chloride \((\text{SO}_2\text{Cl})\). Sulfonyl chlorides react in base with amine groups. For this reason, dansyl chloride is a popular fluorescent “tag” for many amine-containing groups, including those in proteins and on amino acids.

![Figure 21: Dansyl chloride](image)

Other fluorescent tags are available to react with amino groups. These include other tags that have the sulfonyl chloride attachment group. As you prepare for your research, you may be introduced to some of these and given the opportunity to research their effectiveness. Ultimately, you want to contribute to the process of attaching a tag that is responsive to its environment—a sensor tag.

2. **Overview of this laboratory activity**

There are two parts to this experiment. For the first part of the experiment you will attempt to modify the surfaces with drops of dansyl chloride solution. For the second part of the experiment you will take the surface(s) that can be modified and attempt to repeat the dansyl chloride modification by using your stamp.

First, you will examine the ability of dansyl chloride to modify three different kinds of surfaces using drops of dansyl chloride solution. The first one has a reactive group, an amino group, on the surface. The second one has a non-reactive group, a methyl group, on the surface. The third one is a mixture of APTMS and PTMS so it contains both reactive and non-reactive groups.

Second, you will try stamping techniques using the stamp you made last week on paper. Then you will modify the reactive slides using your stamp.
4. **Pre-lab requirements**

You need to come prepared with a detailed procedure to us that should include: procedures for making APTMS, and PTMS slides, amounts to use for the 1:3 APTMS: PTMS ratio, how to make the dansyl chloride solution and . You will need a step-by-step procedure for the different dansyl chloride experiments you will run today.

5. **Materials**

- Staining jar
- Glass slides
- PDMS stamp (from last week)
- 250 mL beaker
- Forceps
- Goniometer
- Dansyl Chloride solution
- APTMS
- PTMS
- Distilled water
- Mohr pipette
- Pipette bulb(s)
- Disposable pipettes
- Sodium carbonate buffer at pH 9.5

6. **Procedure**

6a. **Preparation of slides**
For this week you should make new slides of APTMS and PTMS. You should also create a set of mixture slides using APTMS and PTMS at a 1:3 ratio. For the total amount of APTMS and PTMS you need only a total of 800 μL. Use the same procedure for modifying the slides as you used in week 1.

6b. **Preparation of Dansyl Chloride solution**
To test your stamp you will be doing some fluorescence. There will be a stock dansyl chloride solution in the lab in acetone, an organic solvent that does not react with dansyl chloride. Before use, you need to dilute to the dansyl chloride with a H 9.5 carbonate buffer. This diluted solution must be made fresh every week and needs to be disposed of properly. The concentration of dansyl chloride in the buffer solution should be 1.0 μg / 5 mL. Before coming to lab you should calculate how to do the dilutions to make this solution.

6c. **Suspension of a slide in Dansyl Chloride solution**
You need to test if the fluorescent modification will change the contact angle of the slide. Suspend a slide of each type (APTMS, PTMS, and mixture) in a solution of dansyl chloride. You should use some of the solution prepared in 6b for this. Also, make sure that each slide is not touching other slides, and enough of each slide surface is covered such that taking an accurate contact angle is possible. How long to let the slide remain in the solution is up to you, but you should start with a 10 minute exposure time and then adjust this time if you wish, based on your initial results. Make sure to note how long you leave your slide suspended in the solution. After you rinse your slide with acetone and distilled water pat it dry. Check for fluorescence and note if it fluoresces or not.
Then check the contact angle of the slide to see if it changed from suspending it in the solution.

6d. Modification with dansyl chloride: drops
Use the dansyl chloride solution prepared in 6b on slides. Use a disposable pipette to drop dansyl chloride solution on three different types of slides: APTMS, PTMS, and a mixture slide. Let the drops sit on the slide for 10 minutes. After 10 minutes, rinse the slides with both acetone and distilled water and pat dry with a paper towel. Before you can see the fluorescence you need to expose your slide to long wave UV light. There are lamp boxes for this in the lab. If you do not see them please ask your TA to show you where they are. Put your slide in the box and turn the UV lamp on. Sometimes the fluorescence will be very difficult to see, and be very faint. The UV light is dangerous for your skin and eyes so do not turn it on with your hand in the box, or shine the UV light in your eyes.

6e. Modification with dansyl chloride: stamps
Only attempt to stamp slides that fluoresced from 6d.
Put some dansyl chloride solution on your stamp. You may need to try different ways of doing this before actually attempting to modify your slide. To test this there will be paper that does not fluoresce in the lab available for you to use. Simply try different ways of stamping the paper and checking it for fluorescence before trying to stamp slides. After figuring out a way that works for you, put the stamp on a slide and wait 15 minutes before taking it off of the slide to see the fluorescence. If there is no fluorescence at that time try again waiting a bit longer with the fluorescence solution on the slide. After having placed the stamp on your slide make sure to have something evenly distributing pressure over the entire stamp, i.e. a book, that helps keep the stamp on the slide. Next, remove the stamp from the slide and rinse the slide a few times with both acetone and distilled water. Remember to rinse off your stamp as well so that you can use it in the following weeks.
Before you can see the fluorescence you need to expose your slide to long wave UV light. There are lamp boxes for this in the lab. If you do not see them please ask your TA to show you where they are. Put your slide in the box and turn the UV lamp on. Sometimes the fluorescence will be very difficult to see, and be very faint. The UV light is dangerous for your skin and eyes so do not turn it on with your hand in the box, or shine the UV light in your eyes.

7. Post-lab analysis and results
a) Did the contact angles change after modifying the slides with the fluorescent tag? What does this mean?
b) What effect does the modified surface have on the fluorescent tagging?
c) How does the fluorescence of the different slides (prepared by immersion, drops, and stamping) compare? How can you account for these differences?
d) How well do your images appear, compared to the stamp that you made? Will a change in the stamp make for better stamping?
Laboratory Weeks 4-6 – Research Activity

1. Introduction
So far in this module, you have learned ways to modify surfaces and learned different experiments used to understand how the properties of surfaces change after chemical functionalization. In this activity, you will learn in detail what an assay (see definition below) is and how it works. Furthermore you will continue to be introduced to an exciting area of nanoscale sensors. During laboratory weeks 4-6, you are to use what you have learned in order to answer a research question. The purpose of this section is for you to develop a sensor for the detection of specific ions in a solution. You will develop these sensors specifically to detect transition metal species based upon some of the properties described below.

There are several new opportunities for research in this module. As you will see, the module includes anchoring the fluorescent tag dansyl chloride, to a surface. You may wish to do research on how dansyl chloride-modified slides can be used as sensors. Or, you may have the opportunity to study another dye on the surface. Two candidates are fluorescein, a yellow / green fluorescent system that is best known as the coloring agent used to make the Chicago River green on St. Patrick’s Day, or coumarin, which goes from non-fluorescent to fluorescent when the pH is raised.

Among the research directions you can consider are:

- Changing the ratio of silanes will change the ability of the surface to be modified with a fluorescent group.
- The pH of the buffer used during the fluorescent tagging of a surface will change the amount of fluorescence.
- Other fluorescent tags can be attached to a surface that can be labeled with dansyl chloride.
- Dansyl chloride does attach to some surfaces but does not fluoresce. This may be because of several factors, which can be tested by altering the surface modification.
- Dansyl chloride is sensitive to “quenchers,” including metal ions, making it a potential sensor for these ions.
Appendix A- Glossary

**Adsorption** – The adhesion of a chemical species be it an atom or molecule onto a surface. The adsorbing species is typically present in either the gas or liquid phase while the surface is composed of a solid. Adsorption implies that the atoms or molecules stick onto the surface and do not penetrate into it. The chemical species may stick onto the surface through either chemical bonding (covalent or ionic) or physical mechanisms such as electrostatic, magnetic, hydrogen bonding, or van der Waals forces. It is important to realize that the definition of adsorption is different than the definition of absorption.

**Affinity** – The attraction between particles, atoms, molecules, or substances and other particles, atoms, molecules, or substances. This term is very important in biochemistry since several critical biomolecules display specific attraction characteristics towards other molecules. An example of this can be found in the mammalian immune system where the body creates molecules (antibodies) that display high degrees of affinity towards molecules on the surface of a foreign substance (antigens) such as bacteria or viruses. Another example of affinity in biochemistry can be found in enzyme catalysis reactions. Enzymes are proteins that increase the rates of reactions in the body by decreasing the energy needed for the reaction to occur. Chemists can utilize the affinity of specific biomolecules for a number of applications; in this manual affinity is used for detecting the presence of molecules using surface bound markers that they are highly attracted to.

**Analyte** – A substance that is being measured in a procedure.

**Biochips** – A miniaturized laboratory capable of simultaneously performing a large number of chemical reactions. Biochip development is a young field that is showing potential to measure a number of biochemical events in areas such as genomics (study of nucleic acids such as DNA and RNA), proteomics (study of proteins and their functions), drug discovery, diagnostic detection of foreign pathogens and disease diagnosis.

**Biomimetic Solution** – A solution meant to mimic the analyte levels generally seen in biological solutions. This could be applied for systems containing metals, antibodies, or other biologically necessary species that are screened for using biosensors.

**Biomolecules** – A chemical compound that exists in an organism. This is a generic term that can be used when referring to any molecule found in biochemistry such as nucleic acids, proteins, carbohydrates, lipids, and others.

**Bio-recognition element** – This is typically a non-covalent event leading to the formation of a molecular complex between a host element and a guest element.

**Biosensor** – A platform for the detection of a specific analyte that employs or captures biological molecules. The binding event is transduced into a useable physical property that can be read to determine the presence or lack of the analyte species. The BioCD that
is being created in this manual is an example of a biosensor. The “CD player” produces a different signal when the printed molecules interact with the analyte molecules.

**Chemical immobilization** – The attachment of a chemical species or compound to a surface or matrix so that it cannot move. This reaction can be reversible or irreversible depending on the chemical reaction that occurs. In this manual chemical immobilization of several different chemical species on a number of surfaces is explored.

**Colorimetric** – Relating to the quantitative detection of analytes based upon a color change. Change can be measured by a spectrophotometer, examining the absorption of light at wavelengths related to the species of interest.

**Contact angle** – The angle formed between the liquid/vapor interface of a droplet of liquid on a solid surface. The contact angle is often referred to by the greek symbol theta, \( \theta \). The contact angle is method used to directly measure the energy of an interaction between a liquid droplet and the surface. By maintaining the condition of using the same liquid and varying surfaces this method can be used to effectively measure the wettability of the surface. This material/surface property is directly related to the chemical composition of the material as well as the roughness of the materials surface.

**Covalent bonding** – The sharing of a pair of electrons between two atoms forming a chemical bond.

**Cross-linking** – Cross-link is a term derived from polymer chemistry that is used to describe the linking of one polymer chain to another using a covalent bonding mechanism. In biosensors cross-linking is used to define a molecule (cross-linker) whose purpose is to covalently bind a biomolecule to a surface bound molecule. The cross-linker is in effect a tether or middle man in the mechanism to control the surface adsorption of a specific biomolecule. The cross-linker is chosen such that the chemical reactions it can undergo are specific to certain biomolecules of interest.

**DNA** – Deoxyribonucleic acid (DNA) is the molecule that is used to carry genetic information in any cellular organism. The DNA molecule is a long biopolymer that consists of chemical building blocks which are called nucleotides. Each nucleotide consists of one of four bases: adenine, cytosine, guanine, or thymine. The order that these bases appear on the DNA strand is the “coding” method used by the molecule.

**Hydrogen bonding** – The hydrogen bond is an attractive force that occurs between atoms or molecules that contain partial electric charges of opposite polarity. These forces are important in the structural integrity of several biomolecules, in particular these forces often play very large roles in the stabilization of extremely complex biopolymer structures.

**Labile** – A chemical moiety that can be altered by changing certain environmental conditions. An example would be an acid labile species, meaning one that is removed by treatment with an acid of sufficient strength.
**Ligands** – The term ligand has two distinct connotations in chemistry and biology. In the chemical definition a ligand is an atom, molecule, or ion that donates electrons to a central atom. The central atom is typically a metal atom or ion. The biological definition of ligand typically is used to describe the smaller molecule that is present in a more complex system of molecules held together by intermolecular forces.

**Moiety** – A chemical functionality, specifically a part of a molecule. An example would be to say “chloroform has three chlorine moieties per molecule.”

**Oligomer** – A polymer that consists of less than 10 monomer units. DNA strands that contain less than 10 base pairs, or nucleotides, are often referred to as oligomers, or oligonucleotide which is a term specifically used to describe the DNA polymer.

**Physical immobilization** – Physical immobilization is a method to adsorb molecules onto a surface which relies entirely on physical interactions between the adsorbing molecule and the surface. This method is the easiest way to attach a molecule to a surface but also the most non-specific. Physically adsorbed molecules have random, uncontrollable surface orientations and molecular conformations. For applications were controlled, quantifiable surface reactions are being measured this method is undesirable.

**Protein** – Proteins are complex biomolecules composed of specific sequences to amino acid residues joined together by peptide bonds. These molecules perform important functions in cellular machinery and general cellular processes. There is a large diversity of protein structures in nature. These molecules have a variety of functions and classes which include enzymes used in catalysis, transportation functions of molecules and cellular components, and communication both within a cell and across cells in tissues and organs. These biomolecules are very actively studied; the field of protein study is dubbed “Proteomics”.

**Raster Pattern** – Method of motion where an object will move from one side of an object to the other, down a certain increment, then travel back to the other side. The process is repeated in order to scan a surface.

**Recognition element** – see bio-recognition element

**Schiff base** - A Schiff base is an organic compound in which the nitrogen atom of an amino group is double bonded to a carbon atom. The formation of a Schiff base is an important step in several biochemical reactions.

**Self-Assembled Monolayer** – Self-Assembled Monolayers (SAMs) are single layers of molecules bonded to a solid substrate.

**Silane** – A chemical compound which follows the formula SiH₄. Derivatives of this molecule form reactive species that are capable of forming thin films on glass and silicon based substrates.
**Surface ordering** – Arranging molecules on a surface to have a specific orientation.

**Surface tension** - An increased attraction of molecules at the surface of a liquid resulting from forces of attraction on fewer sides of the molecules.

**Thiols** – Organic compounds that contain a terminal SH functional group at either one or more ends. Follows the form R-S-H, where R is any atom attached to the sulfur atom, typically R represents a hydrocarbon chain. These molecules are of particular importance in the formation of self-assembled monolayer films due to the high affinity of sulfur for several noble metals including: Gold (Au), Platinum (Pt), Silver (Ag), Nickel (Ni), Mercury (Hg), as well other metals.

**Transduction** – A device that is capable of converting one type of energy to another. For example in a biosensor the binding event between a receptor and ligand may be used to generate an electrical signal if an electrochemical cell or electronic change in the material is produced. In this manual the binding event between an antibody and antigen pair on the inkjet modified compact disc surface will affect the height of the printed pattern. This change in height that occurs from binding alters the output of the laser conditions that are measured during the reading of a surface.