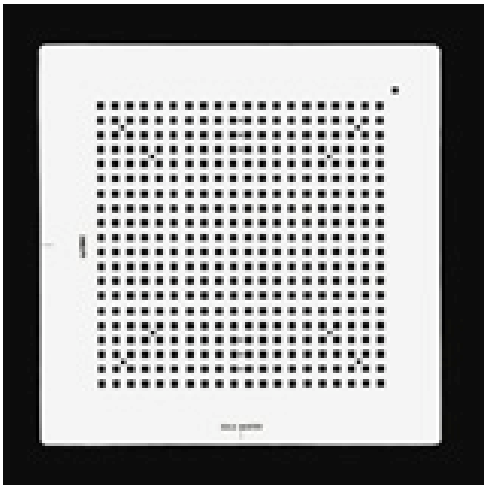

GeneChip® Microarrays

Activity #3 – Manufacturing of GeneChip Microarrays and Building Models

Introduction

GeneChip microarrays are on the cutting edge of technology. They combine knowledge of biomolecules and computer processor manufacturing. They give scientists the ability to access and analyze complex genomic information much faster than anyone could've imagined just ten years ago! What makes them even more amazing is their size. These DNA chips are just over 1 centimeter by 1 centimeter. The manufacturing process for these chips is similar to that used in the semiconductor industry - a combination of chemistry and photolithography. Photolithography is a process of using light to control the manufacture of multiple layers of material.

In this activity, you will learn about the type of process used to manufacture a GeneChip microarray - photolithography. Then, in groups, you will use what you learned to build models of the process out of every day household items. You will present this model to the rest of the class.

Goals

The goals of this activity are:

#1 - To understand the process of photolithography
#2 - To understand how GeneChip microarrays are manufactured
#3 - To illustrate your understanding by building simple models using everyday office supplies and candy!

Procedure

(1) Read the following information on the process of photolithography and the manufacture of GeneChip microarrays. Check and demonstrate your knowledge by answering the question set

(2) View the presentation of the manufacturing of GeneChips at the Affymetrix facility in Sacramento, California

(3) In groups of 3, demonstrate the basic process of photolithographic microarray manufacturing by building a model. Use the 15 - 20 items provided to illustrate your demonstration.

(4) The model (or models) should depict the process of photolithographic microarray manufacturing. You will be given 20 minutes to come up with your idea, build the model and then prepare to demonstrate your model to the class.

(5) Demonstrate your model to the class, explaining how each part illustrates the different steps in manufacturing GeneChip microarrays

Suggested Materials for Model Building (Part 3)

Aluminum Foil	3X5 cards	Flashlight
Pens	Pencils	Paper Clips
Various Small Candy	Glue	Tape
Paper	Pennies	Small wooden blocks
Plastic Wrap	Small Beads	String
Wine Bottle Corks	Pushpins	Rubber Stoppers
Popsicle Sticks	Pipe Cleaners	Scissors

Part 1 – Introduction to Photolithography

Many of the processes for manufacturing GeneChip microarrays were adapted from the semiconductor industry. Using a combination of chemistry and ultraviolet radiation along with minute patterns or stencils, known as "masks," DNA is built onto tiny wafers in a precise sequence. In fact, the steps are so precise, that the entire process can be done in less than 100 steps. Not that amazing? Remember, each chip is about 1.28 centimeters by 1.28 centimeters. Each chip contains sections or "features" that are as small as 5 micrometers by 5 micrometers. And onto each feature are built millions of copies of DNA strands or "probes"! The process is highly automated with the help of computers and robotics and the chips are manufactured in an assembly line fashion.

In this lesson, you will learn the basics of photolithography, the fundamental technique used to build GeneChip microarrays. In order to understand what it is we are trying to manufacture, first imagine a small square of freshly cut grass containing thousands or millions of individual blades of grass sticking straight up. A DNA chip must looks like this from the side but instead of grass sticking straight up, it has short strands of DNA which appear to grow out of a flat glass chip. This DNA is built base by base, from the bottom up, much as grass would grow over time. Eventually, a single chip will end up with millions of 25 base long probes in each feature. Complex? Yes! Can you understand it? Definitely yes!

As a review, DNA is made of molecules known as nucleotides. There are four nucleotides named by the nitrogen base they contain– Adenine (A), Thymine (T),

Cytosine (C), and Guanine (G). Each nucleotide binds together to form a long chain of nucleotides ("a DNA strand"). This chain is also sometimes known as an oligonucleotide. On each DNA strand, the bases point outwards along the chain and it is the other part of the DNA, the backbone, that is created as the sugar group and the phosphate groups combine.

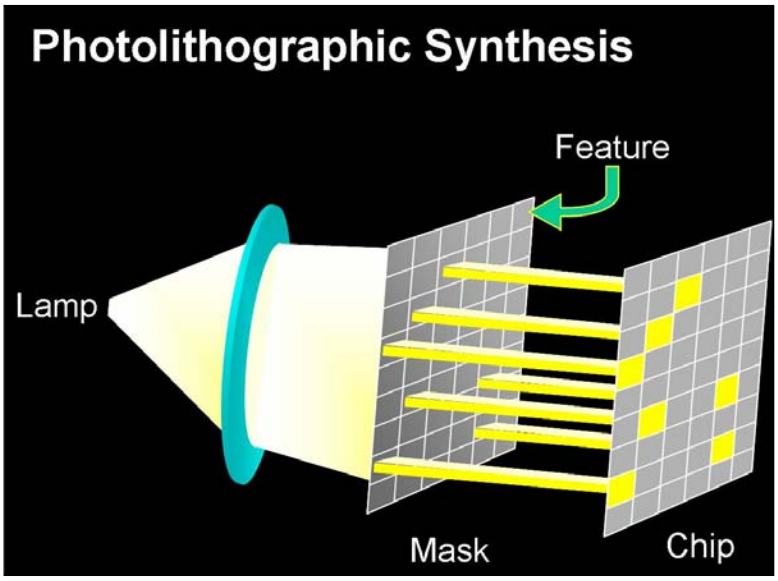

Fig. 1 - The mask only allows light to pass to specific features on the chip

The idea behind manufacturing DNA chips is to, step by step, build single nucleotides into a long chain, sticking each new nucleotide onto the chain sequentially. This process is very precise since it is critical that the nucleotides, or bases, are added in the correct order. By using a mask and ultraviolet light, it is possible to direct this synthesis onto specific features on the chip. The photolithographic mask contains tiny "windows" which are designed to only let light through to the specific features chosen to receive the next nucleotide (much like a stencil). Therefore, it is the manipulation of light that controls the entire process. Light is used to "deprotect" the growing DNA chain and allow another nucleotide to be added. Those chains not "deprotected" will not receive another nucleotide to the DNA strand.

Let's look at the simplified mask on the page below. The diagram represents 20 features (each square) which is only a small section of the whole mask. Remember, there can be over 1.4 million features on each chip and therefore the sections of the mask can be very tiny!

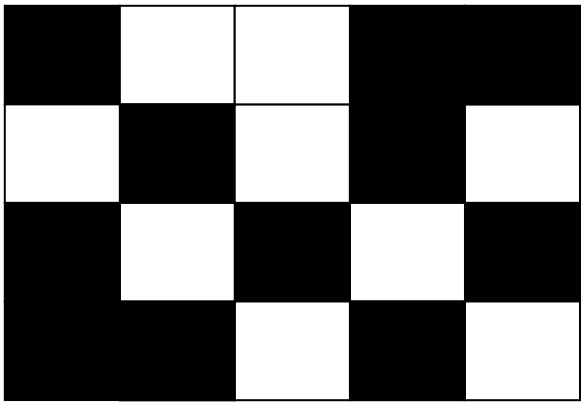

Fig. 2 - A simplified "mask" of 20 features (Top View)

Imagine this mask being placed on top of the chip and ultraviolet light shined through from above. In Figure 2, the black areas are sections which will block the light preventing deprotection of the DNA chains in the features below. This means that those nucleotide chains will not grow in the next step of the process because there will be no activated nucleotides ready to receive the next base. On the other hand, the areas that are not black are areas where light will pass through. Those features will be activated (their nucleotide chains will be deprotected) and the DNA chains will grow with the addition of the next base. This process is repeated over and over again with a new mask each time. Each mask is designed to add new nucleotides to different features.

Part 2 – The Manufacturing Process

Now that you have learned the basic idea behind the process, let's look at it more specifically and with some visuals to help you out with each step. The entire process starts with a glass matrix called the wafer. The wafer is first bathed in silane. The silane (Si) molecule will combine with the glass. The glass is sectioned off into features. Each feature should now be covered in silane molecules. Each silane molecule will provide the starting point for a new DNA strand or probe to be built. The closer together the silane molecules, the more densely packed the DNA probes can be placed.

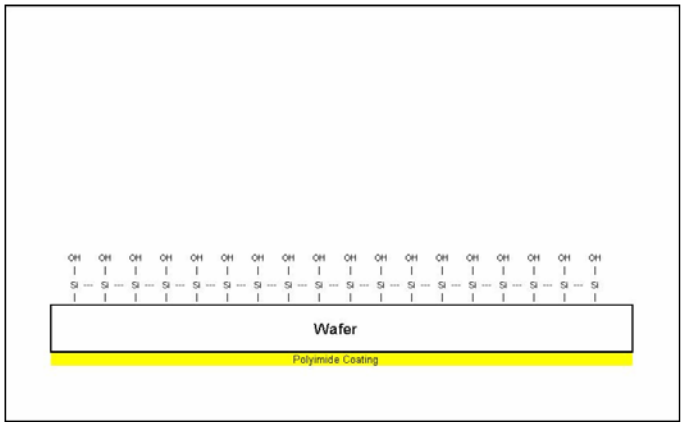

Fig. 3 - Silanation – each "Si" is a starting point

A linker molecule combined with a photosensitive molecule is then added to each Silane molecule. In Figure 4, the linker molecule is represented by the "L" and the small triangle is the photosensitive molecule. The linker molecule serves as the starting point for the addition of the first DNA nucleotide. The photosensitive molecule is a "blocker" or protector molecule. Remember: when this blocker molecule is on the linker or the growing DNA chain, no new nucleotides can be added to the chain, as they will not stick to it. It "protects" the chain from additions

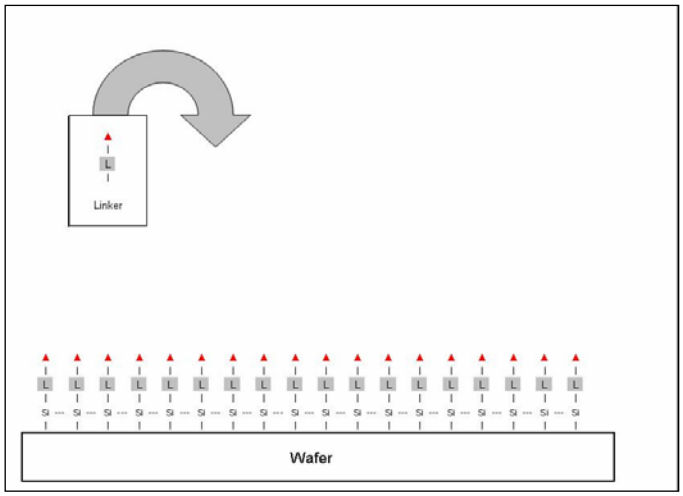

Fig. 4 - The addition of the linker molecule

Once the linker – photosensitive combination molecule has been added, it is time to use the first mask. The mask is placed on top of the wafer with perfect alignment. This is a highly precise activity controlled by trained technicians and computers. In the simplified diagram of Figure 5, 4 features will be shown. (Note, these features are for illustrative purposes only, nothing is really added to the bottom of the wafer.) Once the mask is on top of the wafer, ultraviolet light is shown from above the mask and the features that are exposed to the light lose their protection (note that the triangle molecules are gone from the diagram). These unprotected linker groups are now ready the for the first nucleotide addition.

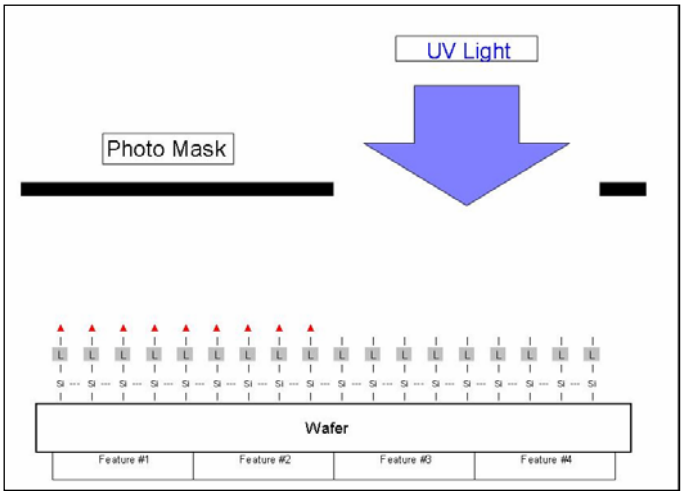

Fig. 5 - Deprotection of Feature #3 and 4

Let's say that, for the probes in these unprotected two features, you want to add Adenine as the first base. The next step is to wash the wafer with a solution that contains single, free Adenine nucleotides that are combined on one side with the same photosensitive molecule as before. This molecule will combine with the exposed linker group at the base portion of the nucleotide. Nothing will combine with the linker groups in the other two features that are still protected by the photosensitive molecules. An adenine has now been added and it is also protected.

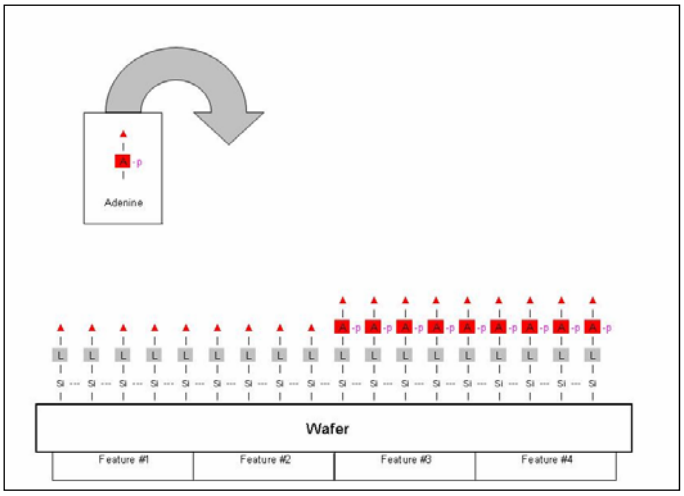

Fig.6 - Addition of Adenine (A) Nucleotides

With every feature protected again, it is time to bring in a new mask to help add the next nucleotide to specific features. This mask may deprotect any combination of the available features—activating either the linker groups on Features #1 and 2 or the adenines on Features #3 and 4 (or any combination of these features). Let's say you want to add a Cytosine nucleotide to Features #1 and #4. You would add a mask that exposes these two features, shine the UV light and deprotect the strands in those features. This is shown in Figure 7 on the next page.

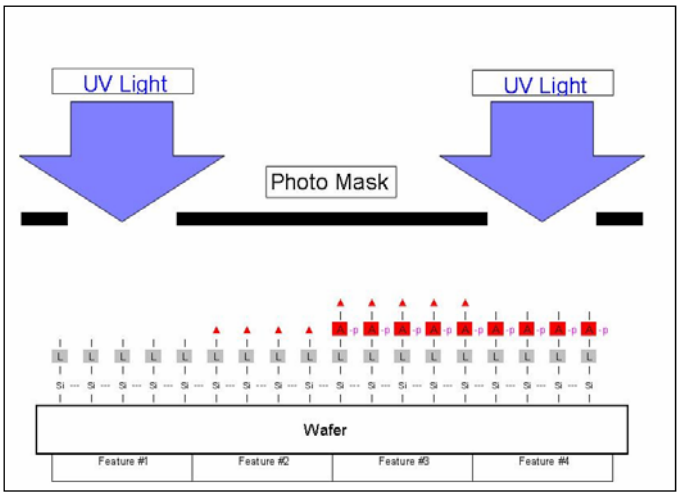

Fig.7 - 2nd mask (deprotecting Feature 1 and 4)

The wafer would then be washed with cytosine nucleotides again linked to the photosensitive molecule. These Cytosine conjugates will bind wherever a deprotected area is found. Notice, looking Figure 8 below, that these nucleotides that are added have a "P" as well as a protective triangle bound to them. These other "protection" molecules prevent nucleotides from binding more than once to each DNA chain, thus avoiding any branched DNA strands. Remember, the DNA probe should be built straight up from the wafer only. The DNA in feature #1 now has a cytosine as its' first base and feature #4 has both an Adenine followed by a Cytosine. The DNA has begun to grow!

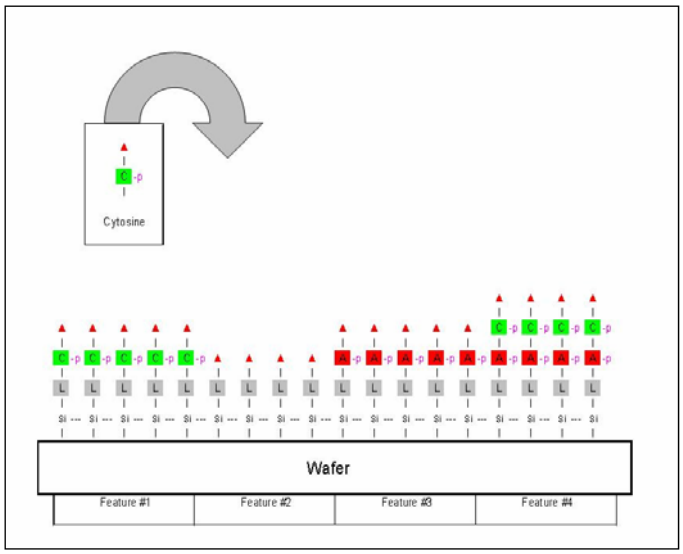

Fig. 8 - Addition of the 2nd nucleotide (C)

If you next wanted to add a Guanine to Features #2 and 3, then you need a mask that exposes these two features (Figure 9). As before, the mask is placed on top of the wafer, the UV light is shined through, and the wafer is washed with a Guanine nucleotide – photosensitive molecule conjugate. The Guanine then binds to the exposed nucleotides. This process continues by repeating these steps over and over again until an entire 25 base long probe is built in each feature. Since there are 25 DNA nucleotides per probe and 4 choices at each step, this usually takes about 100 or fewer steps. Notice that in this process, you are not adding nucleotides to one feature at a time. Rather, the entire

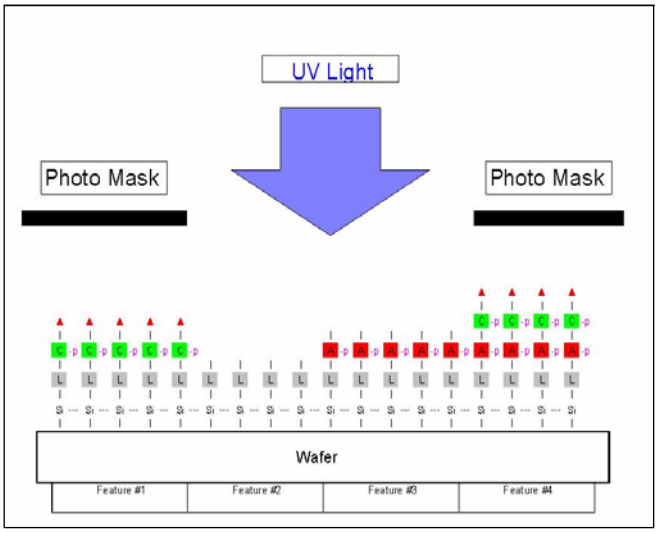

Fig. 9 - 3rd Mask (Deprotection of Feature 2 & 3)

process is calculated perfectly to add one nucleotide at a time to many different features. This way, the process can use many fewer. Keep in mind that these diagrams only shows four features. Some of the newest wafers built have chips with 1.3 million features that are all built at the same time. Adding nucleotides one at a time to individual features would be impractical. Even though there is about 1.3 million features, using photolithography means that it still takes fewer than 100 steps to build every 25 base long probe on the entire wafer.

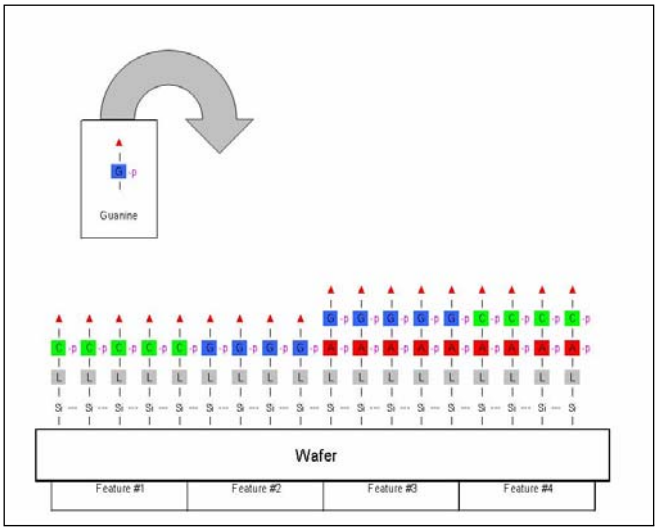

Fig 10 – 3rd nucleotide (addition of Guanine to Features #2 and #3)

Occasionally, a deprotected nucleotide does not bind with the next appropriate base. This is somewhat like a deletion during DNA Replication. A base that should be on the chain is now missing completely. Obviously, if you were to continue this strand, the resulting probes would not have the correct sequence, and would be very different from the others within the feature. To prevent this from happening, an extra step is added just before the addition of each new mask. To prevent an incorrect probe from being made, a "capping" agent is added (see Figure 11 on the next page) which will combine with any unprotected nucleotide. This capping agent is represented by a black box in the diagram to the right. In the diagram, the 2nd probe in Feature #3 did not combine with the Thymine nucleotide added just before. Rather than going back and redoing the addition, the capping agent is

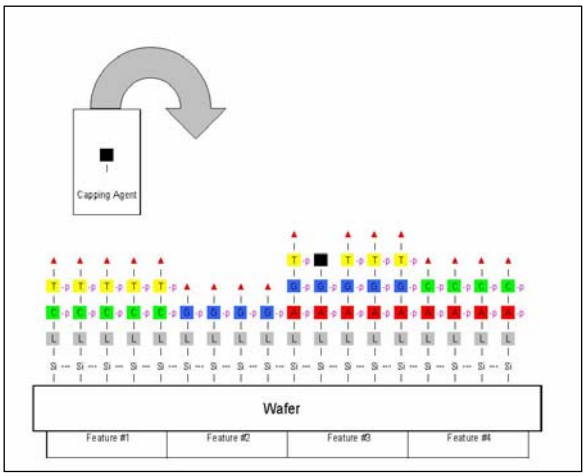

Fig. 11 - Adding of the capping agent

added after each nucleotide wash to prevent any incorrectly built probes from growing any further. In a way, this chain is sacrificed and left incomplete. In the long run, since there are millions of the same probe in each feature, this is not a problem. When the final step is finished, each probe should be 25 nucleotides long, except for those where mistakes occurred and the capping agent prevented their extension. The protection side groups and capping agents are then removed and each and every chip on the wafer is complete.

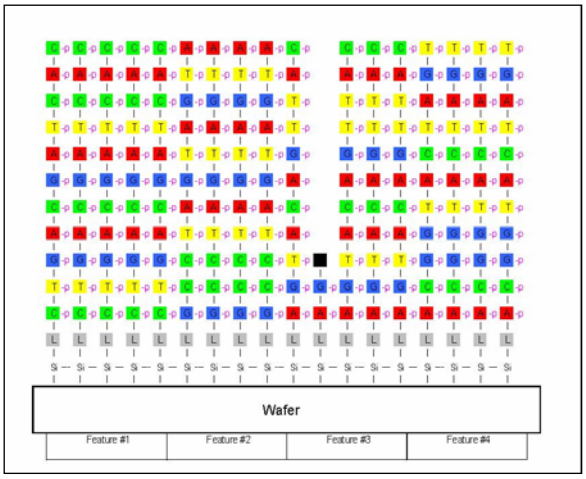

Fig. 12 – Finished Probes

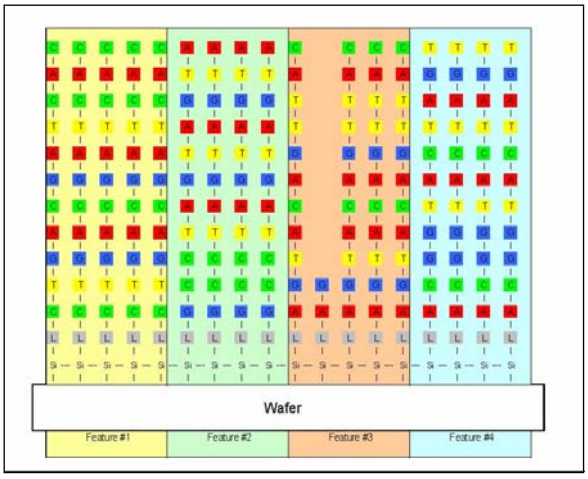

Fig. 13 - Final Results after removal of protection molecule and capping agent

Part 3 – Building in Bulk

Each step in the process is guided by a specific mask used to determine where the added nucleotide will bind. The masks must be very precise and are a major factor in the cost of producting each wafer. Each starting glass wafer is of the same size but it can be divided into many chips. Wafers can be divided into 49, 400, and even 2500 separate chips, depending on how many features you need on each chip. Therefore, the masks must also be divided into 49, 400 or 2500 sections for each chip. Remember that within each chip are millions of features. Needless to say, these are very complex! Once the masks are made, they can be used over and over again. Figure 14 shows a 400 chip wafer-mask.

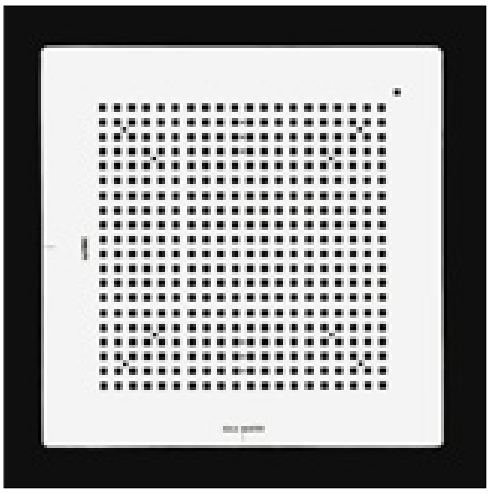

Fig. 14 - 400 chip wafer-mask

The whole manufacturing process is highly automated, though it does require highly trained technicians to control the process. Performed in a clean room in almost assembly like fashion, two robotic machines are used for the two parts of the manufacturing – the chemistry and the photolithography. During the chemistry portion, liquids are added or "washed" over the wafer at an exact point and in the perfect conditions to allow linkage of the molecules. Those free nucleotides that do not combine are flushed out, the capping agent wash is added and the chemistry step is finished. Once this is done, the wafer is removed and placed in the photolithography chamber and the correct mask is placed precisely over the wafer. This step is critical, so special locking marks are used to align the mask perfectly over the wafer. The UV light is shined from above, growing probes are deprotected and the wafer is moved over to the chemistry station to have then next nucleotide washed over it. This is repeated until all step are complete and each nucleotide has been added.

Once the manufacturing process is finished, the wafer is then removed and cut into the smaller sections. Each section becomes a chip which needs to be carefully packaged into a small cartridge and then labeled as the correct array. In each batch, a few chips are tested for quality control purposes to be sure they were correctly manufactured. The chips are then stored or shipped off to research facilities or biotechnology companies using the chips in their work.

Fig. 15 - Packaging the chip

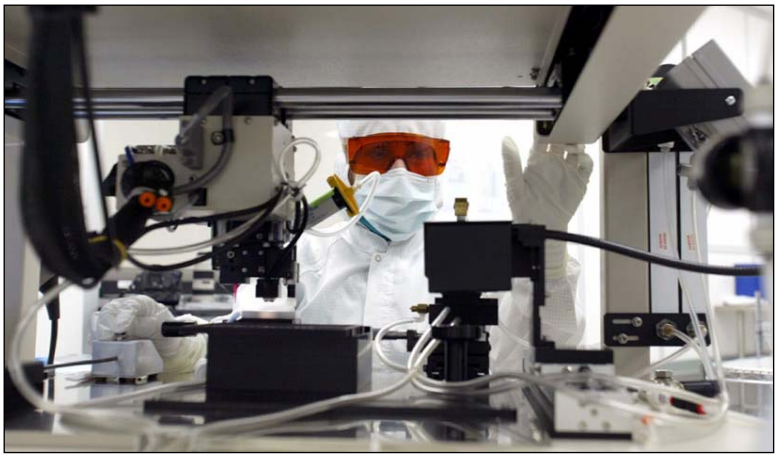

Fig. 16 – Picking and Placing Chips into a Package

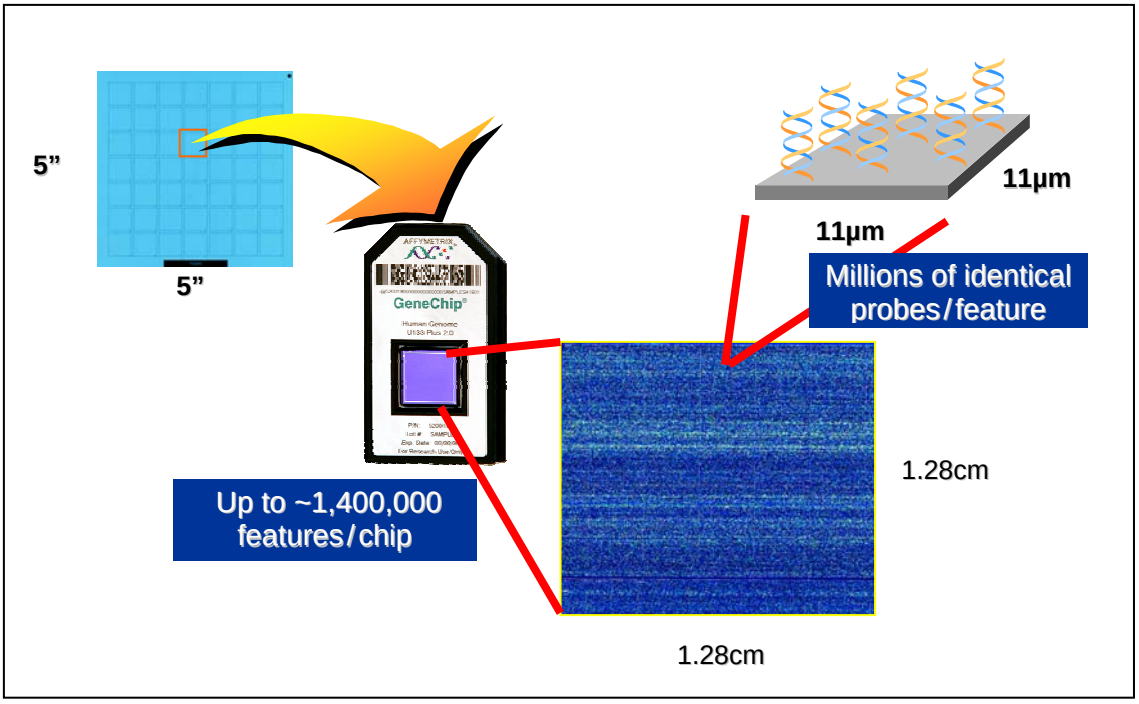

Fig. 17 – Dimension of a GeneChip: Wafer to Chip to a Single Feature