If you were sick, say with cancer, would you not want a drug that has a way of specifically targeting the cancer sell then releasing the medicine without harming the healthy cells? Of course you would. In Dr. Greg Salamo's lab this is exactly what we are trying to do. We are trying to find different ways to transport cancer drugs through the patients body, then release the drugs on the cancer cells and kill them without harming healthy cells. To do this we are examining micro-spheres using atomic force microscopy.

Atomic force microscopy is a form of scanning probe microscopy, that is very high resolution with up to fractions of a nanometer. This microscope was developed by Binning, Quate, and Gerber with IBM and Stanford University in 1986. It is used to make a topographic map of any surface. To make the map a sharp probe is dragged across the surface of the sample, and the interaction between the two is monitored (3, SPM Training Notebook). The atomic force microscope, the Nanoscope V, is a large piece of equipment that easily takes up the corner of the lab in the basement of the physics building. The Nanoscope V can be used not only for atomic force microscopy but also many other things. Since it is a scanning probe microscope it can be used for tunneling atomic force

microscopy, scanning thermal microscopy, along with a list of others including

Illustration 1: Nanoscope V (mbe.uark.edu)

force modulation microscopy. Force modulation microscopy is mostly what we use it for.

The atomic force microscope works using interactions between a tip, cantilever, laser, scanner, and computer. Illustration 1 shows the set up of the atomic force microscope in the physics building lab. The big cream colored box contains everything but the computer. This box can control the heat of the system when closed. They all work together in order to give an image of the sample's surface. Since the atomic force microscope is such a versatile system there a few different modes it can operate in; tapping mode, contact mode, and non-contact mode. Illustration 2 is how the system works in tapping mode. A laser is sent through the system to the cantilever and tip system giving the reading in the computer of the sample based off of angle measurements. Then

those measurements are interpreted by the computer to give an image of the surface of the sample.

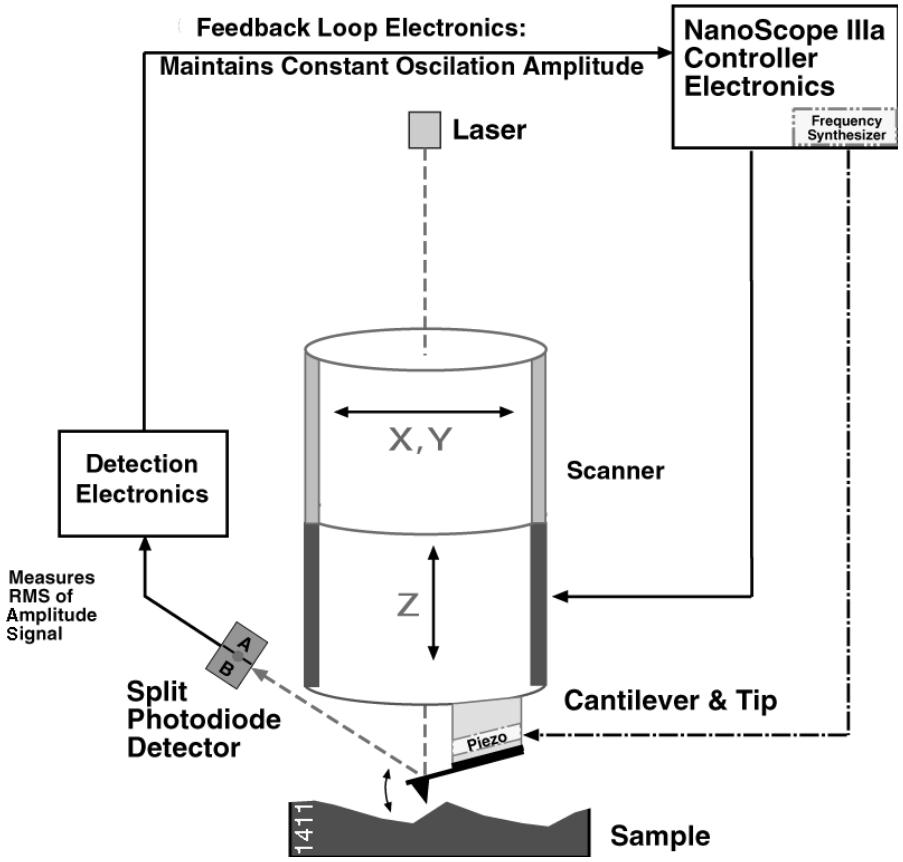

Illustration 2: Feedback electronics (Veeco)

This process is common in all three of the a fore mentioned modes. What is different between the three modes is the movement and placement of the cantilever and tip system. In tapping mode the cantilever and tip system is oscillating up and down with an amplitude between 20 and 100 nanometers (10, veeco). This means that the cantilever has to be lifted slightly higher in the

system then in the other modes as to not break or bend the tips when making contact with the surface of the sample. In contact mode the tip is always in contact with the surface of the sample. This gives a quicker scan time then the tapping mode, especially on rougher samples. In non-contact mode the tip never comes into contact with the sample surface but oscillates above the surface with and amplitude of a few nanometers (11,Veeco). With all three modes there are advantages and disadvantages to each. As stated before contact mode is better used for quick scans of surfaces, where tapping mode is better used for samples that are easily damaged or if a clearer image is needed. Non-contact mode is good when wanting nothing to touch the sample. With the advantages lie disadvantages as well, with contact mode the image can be distorted due to the force of the contact between the sample and the tip. In tapping mode the scanning time is longer because it has to make several traces in order to get the same quality image as in contact mode. Finally, non-contact mode works well only with very hydrophobic samples, making the range of materials used much smaller (13, Veeco).

For different samples different kinds of tips and cantilevers can be used. For each of the three modes there are different tips, and within the range of tips for each mode there are different spring constants for the tip and cantilever system. The higher the spring constant the smaller the deflection or larger the force depending on the relationship due to Hooke's Law. As a tip ages different things occur that could impact the image that is taken by the microscope. Illustration 3 shows what an image would look like if the tip is split from previous uses. Where illustration 4 shows when a tip is moving too fast and is not picking up the exact shape of the surface of the sample. These can both be easily avoided; the double tip image can be avoided by replacing the tip when this image first appears, the flying tip image can be fixed by slowing the scan speed of the image as well. Another issue that occurs when scanning samples is called non-tracking. This is when the tip is too big or not sharp enough to read the

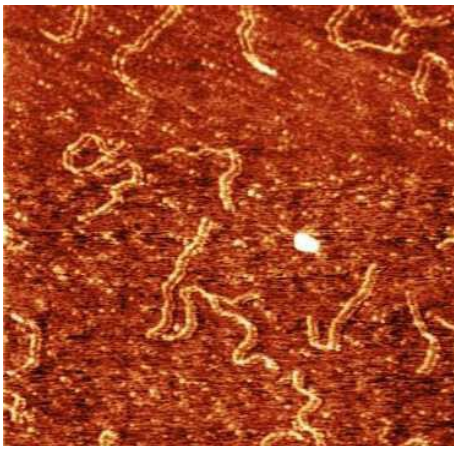

Illustration 3: Double Tip (veeco)

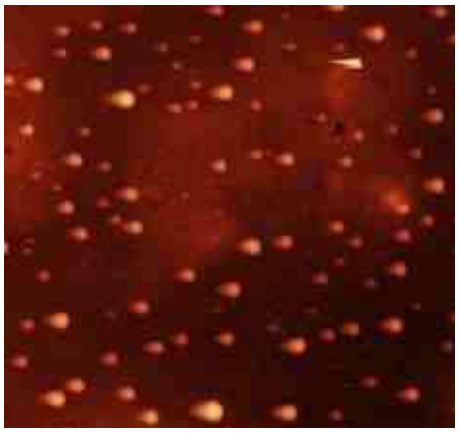

Illustration 4: Flying tip (veeco)

surface of the sample clearly. When this happens pictures will be blurry or unrecognizable. To fix this issue the tip should be changed to a small or less blunt tip. These are only three examples of artifacts that can occur in imaging. The others are all just as easy to identify and correct the issue. Earlier different types of cantilevers and tips were discussed, it is assumed that all cantilevers must have tips, this assumption is incorrect.

When working with micro-spheres and micro-bubbles a tip-less cantilever is used to gain force versus distance curves, to determine the mechanical properties of the hollow micro-spheres (Langmuir). A micro-bubble is an extremely small bubble that can be suspended in a liquid such as blood. This ability to be suspended in bodily fluids like blood makes the micro-bubble ideal for drug delivery. Micro-spheres and bubbles also have large surface areas with a negatively charged surface making the bubbles easy to attach positively charged ions to (Riverforestcorp).

To determine the mechanical properties of micro-bubbles the tip-less cantilever is used as a probe against the micro-bubble. Illustration 5 shows two different placements of the cantilever against the micro-bubble. When placing

the cantilever in the first image it is directly centered on the micro-bubble so that all pressure is uniform over the bubble. In the second image it is on the very edge of the micro-bubble so that it applies pressure only to that one side. This is done to determine if the micro-bubble is uniformly stable or only stable at certain points. Different cantilevers with different spring constants and micro-bubble with different diameters and shell thickness are used to make the curves in order to determine what properties are best suited for drug delivery.

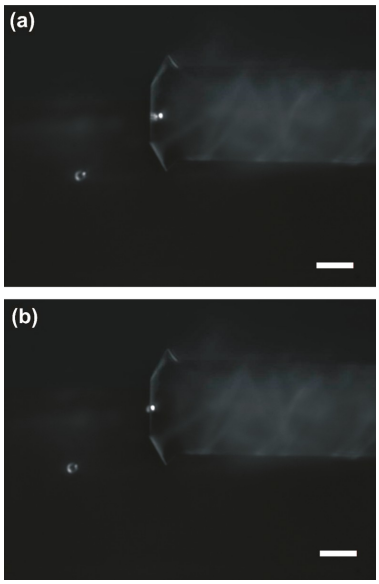

Illustration 5: a) Micro-bubble under tip-less cantilever b) cantilever at edge of micro-bubble (Langmuir)

In an experiment conducted by researchers at the University of Endinburgh, it was determined that after several force curves with the same

micro-bubble and cantilever that permanent deformation begins to occur within the micro-bubble.

Why is the force the micro-bubble can with stand important? It is important because knowing the force needed to destroy the micro-bubble gives researchers the ability to determine exactly how useful the micro-bubble can and will be in delivering drugs. Since drugs can be encapsulated by the micro-bubble it is important to determine how to release the drug when it reaches the infected cells. Ways that this is already being done is through ultrasound-mediated micro-bubble destruction (Cardiovascular Ultrasound). For this they insert the micro-bubble and drug into the body and wait for it to reach the cancer cells and then send a shock of ultrasonic waves through the system destroying the micro-bubble, releasing the drugs. The issue thus far with this method is that the released drugs may not actually be attacking the sick cells when they are released before entering the cell, thus still harming healthy cells. This ability to destroy the micro-bubbles through ultrasound has led to others wondering if it is possible to destroy them other ways, thus why experiments are being conducted on how much force and how long it takes to destroy a micro-bubble.

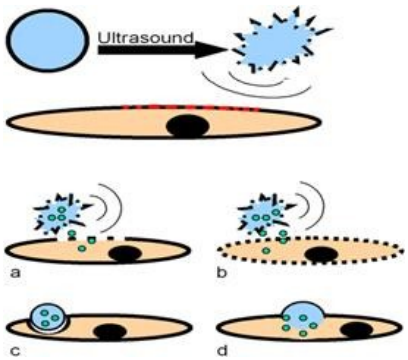

Illustration 6: Destruction of microbubbles by ultrasound resulting in increased membrane permeability by shear stress, temperature rise and activation of reactive oxygen species, Drug delivery from microbubbles is by a) transient holed induced by shear stress b) increase in membrane fluidity c) endocytosis of microbubbles d) fusion of the microbubble membrane with the cell membrane (ultrasonic micro-bubbles)

Another method for transporting drugs is to attach them to the outside of the micro-bubble and then get them to release due to the fact that micro-bubbles bind to damaged vascular endothelium. Illustration 7 shows this idea with ligands attached to the surface of the micro-bubble. This means that they are drawn to damaged cells and then deliver drugs to those specific cells without any other equipment needed, such as ultrasound waves (Cardiovascular Ultrasound).

Thus far only destroying the micro-bubbles with ultrasound has been discussed. Micro-bubbles can also be moved through membranes using ultrasounds of different strengths. This is helpful when trying to move drug

covered micro-bubbles through the membrane of the cell so that it can deliver possible life saving drugs. Because it is possible to move micro-bubbles without destroying them, means that it is possible to move the drugs from the entry location to the site of cancer cells without leaving drugs through out the body along the way. Thus meaning lower concentrations of the drugs can be used to kill the same size cancer cells as before.

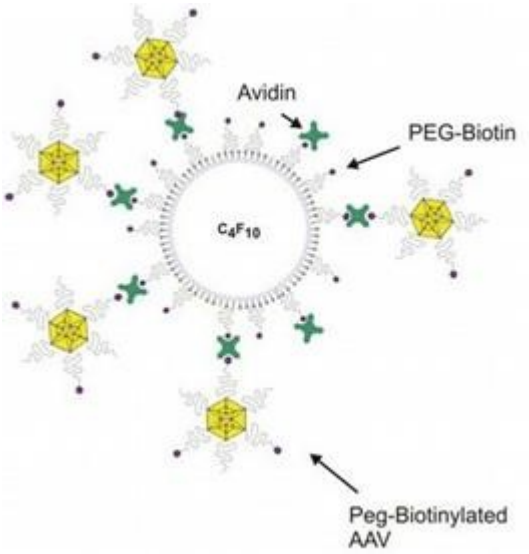

Illustration 7: Micro-bubble with ligands on surface (ultrasonic micro-bubbles.)

In the future this research could lead to multiple opportunities that could lead to new ways in treating cancer and other medically challenging illnesses. Micro-bubbles are most promising in the line of cancer research for their ability to hold and release drugs and their ability to have drugs attached to them. If

other ways of destroying the micro-bubbles can be discovered then this will lead to a whole new realm of possibilities.

References

"Full Text | The Use of Microbubbles to Target Drug Delivery." *Cardiovascular Ultrasound*. Web. 21 Feb. 2010. <http://www.cardiovascularultrasound.com/content/2/1/23>.

Glynos, Emmanouil, and Vasileios Koutsos. "Nanomechanis of Diocompatible Hollow Thin-Shell Polymer Microspheres." *ACS Publications*. Langmuir Article, 20 Apr. 2009. Web. 19 Feb. 2010.

"Microbubble - Definition of Microbubble by the Free Online Dictionary, Thesaurus and Encyclopedia." *Dictionary, Encyclopedia and Thesaurus - The Free Dictionary*. Web. 19 Apr. 2010. <http://www.thefreedictionary.com/microbubble>.

"Micro-Bubbles - Chemical Free, Environmentally Friendly Technologies - Micro Bubbles, Nano Bubbles, Micro-Nano Bubbles, Solar Powered Pond Water Cleaning System"" *Riverforest Corporation - Chemical Free, Environmentally Friendly Technologies - Micro Bubbles, Nano Bubbles, Micro-Nano Bubbles, Solar Powered Pond Water Cleaning System"* Web. 19 Mar. 2010. <http://www.riverforestcorp.com/micro-bubbles.html>.

"Ultrasonic Microbubbles: A New Vista In Drug Delivery And Medical Imaging | Pharmainfo.net." *Pharmaceutical News, Pharmaceutical Articles, and Pharmaceutical Jobs for You ! | Pharmainfo.net*. Web. 10 Feb. 2010. <http://www.pharmainfo.net/reviews/ultrasonic-microbubbles-new-vista-drug-delivery-and-medical-imaging>.

Veeco. *A Practical Guide to Scanning Probe Microscopy*. Veeco Instruments, 2005. Print.