Lesson Plan

Title How to make measurements of living materials as they interact.
Primary Subject Area **Physics and Biology – Optics Interference, Energy Conservation and Antibody-Antigen Interactions**
Grade Level 11 and 12
Overview In order to measure the interactions between antibodies and antigens, a series of light rays are shone on a platform of antibodies and antigens. As the light rays reflect from the surfaces, the reflected rays interfere with each other. The amount of interference which occurs depends upon the pathlength difference of the paths covered by the two rays. From these measurements, it can be determined what the depths of the antibodies and antigen levels are. From these measurements, the amounts of mass present in the antibody and antigen levels may be calculated. **Then, by subjecting the substrate to a laser beam and by utilizing the principle of energy conservation, it is possible to utilize and spectroscopy evaluation to determine the chemical materials involved in the antibody-antigen interaction.**
Approximate Duration 2 – 4 class periods
MA Framework Biology – 1.1 and 1.2 (on the chemistry of life), 4.7 (on cell communication). **Introductory Physics - 2.1 (Conservation Energy), 4.1 (Wave Characteristics), 4.4 (Reflection and Refraction of Waves), 6.2 (Electromagnetic Spectrum)**
Interdisciplinary Connections The wave and energy principles of light are being used to discern how biological systems operate.
Lesson Objectives Students will be able to apply the principles of reflection, refraction, and interference to discern how much mass of antigen interacts with an amount of antibody material. Furthermore, they will gain how you can apply the principle of energy conservation to discern the type of material involved between a laser beam and a target composed of layers of antibody and antigen materials.

Lesson Materials and Resources Leading to this lesson, many labs and demonstrations will be performed to learn the topics covered in the background information. For this lesson, background information will be provided to explain how the immune system creates the antibody and antigen system. Then, a discussion will ensue, using a powerpoint presentation, to explain how the Interferometric Reflectance Imaging Sensor (IRIS) is used to measure the amounts of antibody and antigen materials interact with each other. Then, a discussion will ensue in how the Matrix Assisted Laser Desorption Ionization (MALDI) mass spectroscopy technique is used to determine the actual chemical materials involved in the antibody-antigen interaction. Demonstrations will be performed using slinkies; two pyrex beakers (100 ml and 250 ml), glass stirring rod, and corn oil.
Technology Tools and Materials Laptop computer with a Powerpoint-Quizdom presentation utilizing a remote clicker system. Mercury lamp with four filters, two short glass plates, micrometer, and metric ruler.
Background Information Previously, students will learn about basic wave properties such as the ability for wave energy to pass through another wave without affecting each other. When two waves meet at a certain point in a medium, the medium responds to both waves and displays the properties of constructive and destructive interference. When waves pass from one medium into another, wave energy is partially transmitted between the media and partially reflected from the border of the media. For electromagnetic waves, the amount of wave energy reflected depends upon the difference in optical density of the two media. From the study of Young's Double Slit experiment, students will learn about the importance of the pathlength difference between two light rays to determine the wave interference between waves. The index of refraction of a material permits scientists to determine the wavelength of light as it passes through the material.

Lesson Procedures The lesson opens with the question, "How do you make measurements within a living system to discern the operation of biological particles which are the size of nanometers ?" This is followed by a discussion concerning the operation of the human immune system focusing on the roles of antibodies and antigens.

We now focus on the construction of the IRIS system. During this discussion, the students will be provided some new information concerning the optical properties of living matter. In particular, that the index of refraction for the antibody and antigen are both equal to 1.45. This is also the index of refraction for silicon dioxide. A demonstration is now performed in which the glass (n= 1.5) stirrer, and the two pyrex beakers are placed inside each other. Then, the corn oil (n = 1.45) is poured into the small beaker surrounding the stirrer. The students are shown how they can see the reflected image of the stirrer even though the optical densities are almost equal. The corn oil is poured in so that it overflows between the two beakers. Here, the students see that no light reflects from the inner beaker.

This leads to the students discussing in small groups what will happen to the light rays emitted by an LED placed over the target of antigen, antibody, silicon dioxide and silicon. They will further go into discussing what will determine the pathlength difference of the resulting rays. This is followed by a discussion concerning the advantages of repeating the experiment with different wavelengths of light.

This is followed by a lab exercise in which the students create an air wedge between two glass sheets by placing a strand of hair between them. They will then shine a mercury lamp's filtered light onto the top of the glass plates. By doing an analysis similar to the one they had done for the IRIS system, they will make four measurements of the hair's thickness. The four measurements are distinguished from each other by the use of differently colored filters.

The final step of this will be a description of the use of the MALDI system to produce a spectrum analysis of the target sample. The students will explain how an understanding of the application of energy conservation could be used to determine the mass to charge ratio of the discharged samples.

Assessment Procedures Students will work in small groups to determine how the IRIS and MALDI systems are used to determine the size and compositions of the interacting antibodies and antigens. Each group will submit their solution before we undergo the class discussion.

Each individual student will submit a lab report , including underlying description of the pathlength difference and interference technique used in the hair measurement lab.

Accommodations/Modifications With students who have difficulty with the mathematics aspects of this program, less emphasis will be placed on discerning the actual pathlengths and pathlength differences of the different systems. This will left in general terms so that the students can interpret the resulting light and dark lines seen in the hair thickness labs to correspond to pathlength differences without going into great detail in determining the overriding equations. The emphasis will be placed on the conceptual aspects of the experiments. There will be an increase in the number of demonstrations used to permit the students to picture what is happening.

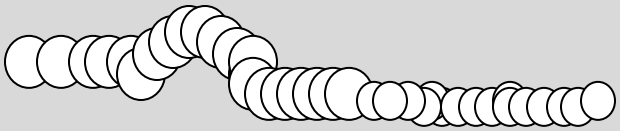
Reproducible Materials The following demonstrations and lab can be used :

Demonstrations of Wave Properties Used in the IRIS

Equipment : Large Slinky 2 glass plates (approx. 3'x10'x0.25') Mazola Corn Oil
Long, tight coiled spring Small mercury lamp w/ colored filter pyrex stirring rod
Pyrex 100 ml beaker
Pyrex 250 ml beaker

Property - As wave moves between media, energy is partially transmitted and partially reflected.

Demo - Connect slinky and spring together on floor. Stretch sliky out and send single pulse down it. Note what happens as pulse hits the spring.



Property – The nature of the reflected energy pulse depends upon the relative natures of the two media (for mechanical waves – its rigidity. For light, the optical densities)

Demo - With a stretched slinky, have one person hold one end rigidly as the person on the other end of the slinky sends a single pulse toward him. Note the reflection of the pulse from the second person's hand.

(Optional) Demo - Tie a long, loose string to the end of the slinky. Send a pulse down the slinky toward the string. Note the reflection from the string.

Question - What would happen if a wave hits a material of equal rigidity (optical density) to the one that it was originally traveling through ?

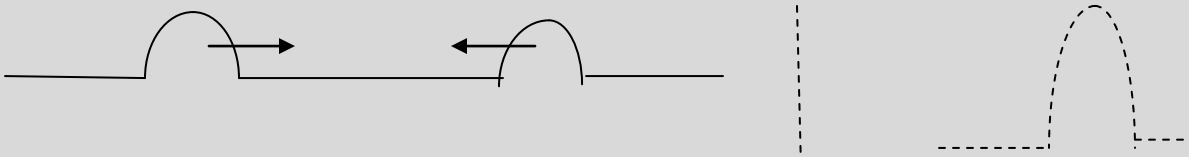
Demo – Show pyrex stirrer inside 100 ml beaker inside of 250 ml beaker. Note that you can see through each beaker and see the separate reflections of light from each pyrex surface as the light moves from the air into the pyrex. Pur Mazola oil into the 100 ml beaker and see how the stirrer appears to "disappear". Let the oil overflow from the 100 ml beaker into the 250 mll. See how the 100 ml beaker seems to disappear. Note that the index of refraction (the optical density) of the oil is the same as that of the pyrex. The light does not "sense" a change in media.

Property - Waves pass through each other unchanged rather than bouncing off from each other.

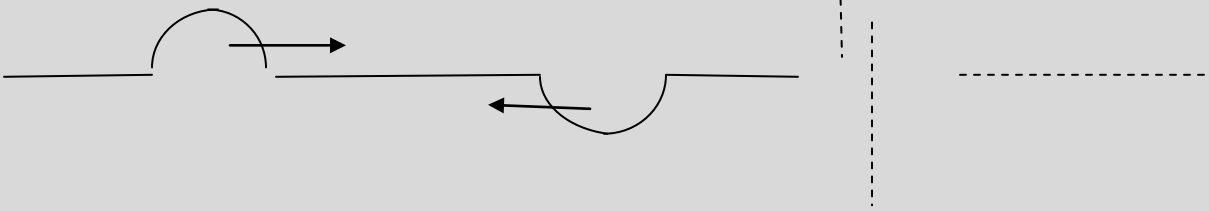
Demo - On the floor, have people on opposite sides of a slinky send pulses toward each other – one pulse is small and the other is large. See if the pulses pass through each other or bounce off from each other.

Property - Wave Interference.

Demo – On floor, have people on opposite ends of the slinky send pulses toward each other on the same side of the slinky. Note what happens to the slinky at the point at which they meet.

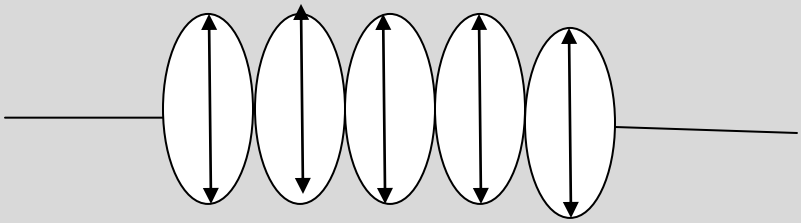


Demo - On the floor, have people on opposite ends of the slinky send pulses toward each other on opposite sides of the slinky. Note what happens to the slinky where they meet. Have students compare results to the last demo. It is hard to see the destructive interference.



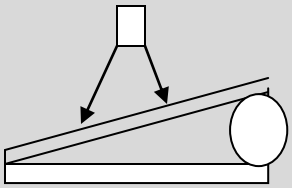
Property – Identical wave interference generating a standing wave with nodes and antinodes.
Demo – Students standing 10-15 feet apart holding ends of tightly coiled spring. One holds the spring firmly as the other sends a single pulse down the spring. Note the nature of the reflected pulse. Note that if you send a series of pulses down one side (creating a wave motion), there will be an equivalent reflected wave moving from the other end of the spring. These two waves will create an interference pattern.

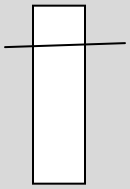
Demo - Using the same set up as before, have the student send a continuous series of pulses down the spring. Note that the incoming and reflected waves interfere with each other forming a standing wave with nodes and antinodes.



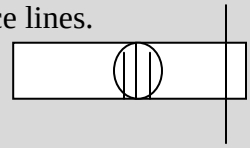
Property – Light creates a wave interference pattern with nodes (dark) and antinodes (bright) lines that resembles the pattern seen before.

Demo - Place glass plate on top of white paper. Place hair across the end of the plate. Place the second plate on top of the first plate.

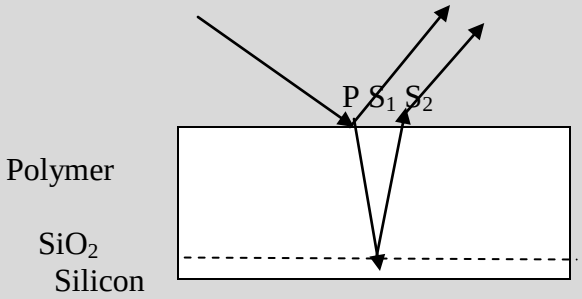




Over the plates, shine a mercury lamp down onto the plates. Look at the light bulb's reflection from the surface to see the interference lines.



This can be done as individual labs by the students or, with the aid of a document camera, this can be shown to the entire class.
At this point, the students should have an understanding of the wave properties involved in the IRIS system lab.



(Note that no refraction or reflection occurs between the polymer and SiO_2 layers since they have equal optical densities.)
Pathlength Difference = PD = PS_2 - PS_1
In order to form an antinode, the pathlength difference must equal a whole number of wavelengths.

$$PS_2 - PS_1 = m\lambda \qquad m = 0, 1, 2, 3, \ldots$$

In order to form an node, the pathlength difference must equal an odd number of half wavelengths.

$$PS_2 - PS_1 = (m + 0.5)\lambda \qquad m = 0, 1, 2, 3, \ldots$$

For other pathlength differences, the type of interference pattern varies between these two extreme values. Correspondingly, the relative brightness of the resulting reflected light varies between dark and very bright.
Example of what the waves look like. Consider there being two identical rays coming toward point P on the top plate. One of the rays bounces from the plate at P while the other goes through the polymer and SiO_2 layers and reflects from the silicon layer.

Correlating the pathlength differences that we find for each of the different colors of light (blue, green, yellow, red) which have different wavelengths, we can calculate the thickness of the height of material above the silicon layer. If we do this for each of the separate layers and then take the difference in heights between levels, we can calculate the height of each layer of material. This height measurement will also correspond to the amount of mass which is in the layer of material.

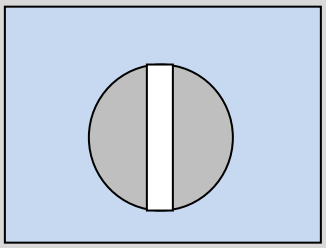
Air Wedge Lab : Using wave interference to measure hair thickness

Equipment : mercury light source
2 meter sticks
diffraction grating
2 glass plates
1 meter stick stand
micrometer

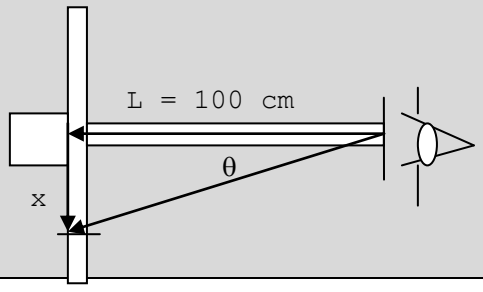
Procedure : 1. Use the procedure from the Young's Interference Experiment to determine the wavelength of the dominant light color (major color seen) of the provided light source or use the provided value.

N = number of slits/cm = _____ d = _____ cm/slit

a. Place a single color filter in front of the mercury light. Then, put a piece of paper in front of this light which has a vertical slit cut in it.



b. Place a meterstick straight out in front of the mercury lamp. Place another misterstick directly in front of the light which is perpendicular to the first meterstick. The light should be at the 50 cm mark on the meterstick.



c. Sit at the end of the first meterstick and look through the diffraction grating toward the mercury lamp. Look for a bright colored light strip to the left or right of the lamp. Tell your partner where along the second meterstick the light strip appears to be located. Then, measure the distance (x) between the light and the colored strip.

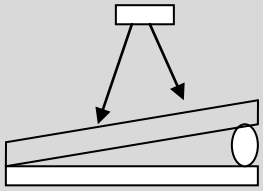
d. Calculate the angle of tilt between the first meterstick and the line connecting the person's eye and the location of the light strip. $\theta = \tan^{-1}(x/L) = \tan^{-1}(x/100)$

e. Use this angle to calculate the wavelength of the mercury light.

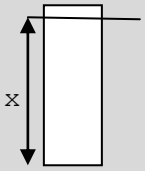
$$\lambda = d \sin \theta$$

color	L	x	tan θ (x/L)	θ	sin θ	wavelength d sin θ

2. Place a tightly held, straight hair between 2 glass plates near one end of the plates.



side-view



top-view

3. Measure the distance (x) between the hair and the far end of the glass plates. x = _____

Remove the slit from in front of the light source, but leave the glass plate in place in front of the light. This is necessary to block out the ultraviolet rays emitted by the light from reaching you.

4. Shine the lamp onto the plates from above. Move the light until "shadow" (interference) lines are easily seen crossing the plates.

5. Locate the point which is halfway between the hair and the end of the plates. Count the number (N) of dark lines to be found within 1.0 cm of this point.

N = _____

6. Between each interference line there is an increase of $\lambda/2$ in separation of the two plates. Therefore the thickness of the hair can be calculated by :

thickness = (lines/cm)(cm/plate-length)(thickness change/ dark line)

thickness = N x $\lambda/2$

thickness = $\frac{N \times \lambda}{2}$ = _____ cm

7. Place the hair between the two arms of the provided micrometer and close the arms. Measure the thickness of the hair. To find the actual

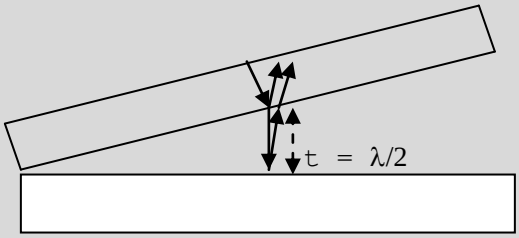
thickness multiply the number read on the barrel-scale by 1 x 10^{-5} meters.

thickness = _____ x 10^{-5} meters

Questions :

1. Compare the two thicknesses. How do you account for the discrepancies ?

2. Explain why there is an interference line when the two rays shown below interfere with each other.



3. If the thickness of the air wedge increased by half a wavelength, explain why another interference band occurs.

4. Explain why the point where the two glass plates come together always has a dark band.

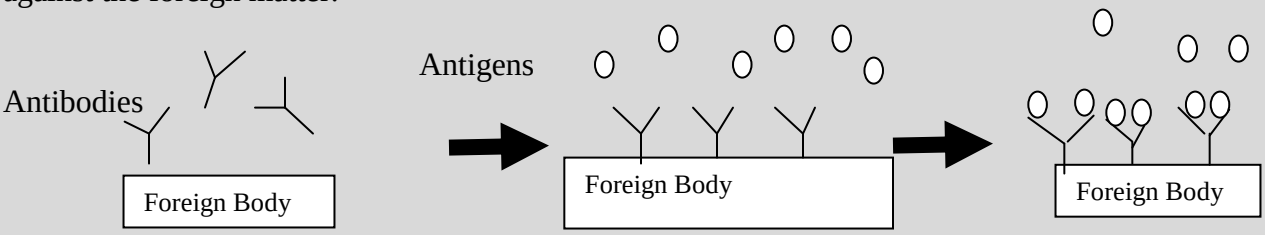
Measurement of Human Immune System Operation

Name _____

Biology Background:

There are many interacting aspects working during the operation of the human immune system. We will focus on how to make measurements of the antibody-antigen response.

Antigens are the proteins that are introduced by organisms foreign to a body (i.e. virus or bacteria). Antibodies are produced by the body as a response to the foreign organism. Each antibody is matched in nature to a specific antigen. Where the antibody bonds to the specific foreign material, the antigen then bonds to the antibody. The antigen, then, is designed to operate against the foreign matter.



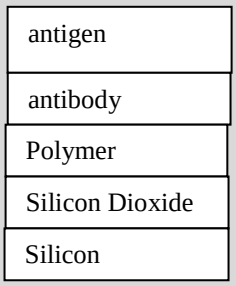
In the field of medicine, detection of these antigen and antibody particles are often used for diagnostic purposes. Since they are related to the invading foreign matter, being able to identify and measure the quantities of antibodies and antigens present may serve in determining the nature of the foreign matter. Detected antibodies and antigens may provide information about the disease infecting the body and its progress. Some examples of antibody arrays include detection of biohazards, bacterial infections, and biological markers for cancer research and diagnostics.

The question to be raised at this point is "How can we make measurements of particle systems which are only nanometer (10^{-9} meter) lengths in height ?" It is important to remember that the wavelengths of visible light fall in the range of 400 to 700 nanometers.

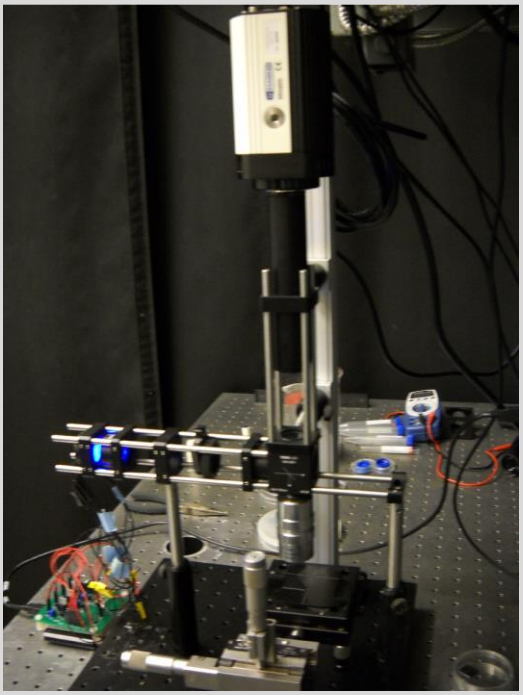
What we will look at now is one possible solution to this problem currently being studied at the Phototonics Laboratory at Boston University. The systems being utilized are referred to as the IRIS (Interferometric Reflectance Imaging Sensor) and MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight) systems.

I. IRIS Measurement:

The IRIS system is composed of a layered structure. On the base of this structure is a silicon material. Layered above this are silicon dioxide, a polymer, the antibody under study and the antigen.

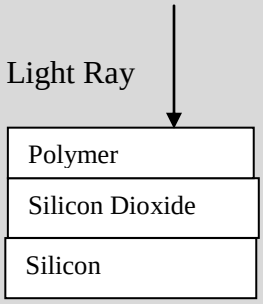


A wavelength controlled light source is used to shine four different colors of light onto this target. The reflected light from this set of surfaces is then captured by a CCD camera which is connected to a computer system.



The proteins making up the antibody and antigen are found to have equivalent indices of refraction equal to 1.45. The polymer and SiO_2 have been chosen to form a system that will join the antibody to the silicon structure but cause no change in the optical density of the system. Both the polymer and the SiO_2 have indices of refraction of 1.45.

A series of measurements are made with this system. First, the silicon, SiO_2, and polymer are placed on the stage of the IRIS and the light of wavelength λ passes through air as it is is shone onto it.

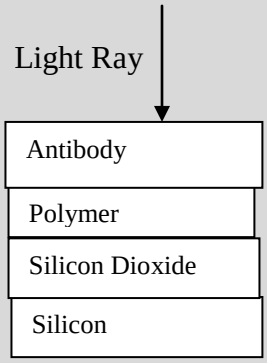


1. What happens to the light ray when it reaches the surface of the polymer ? Is the light reflected and/or transmitted at this interface ? If there is a reflected ray, is it reflected inverted or erect from this surface ?

2. What happens to the light ray when it reaches the surface of the silicon dioxide ? Is the light reflected and/or transmitted at this interface ? If there is a reflected ray, is it reflected inverted or erect from this surface ?
3. What happens to the light ray when it reaches the surface of the silicon (note that silicon is not a transparent or translucent material)? Is the light reflected and/or transmitted at this interface ? If there is a reflected ray, is it reflected inverted or erect from this surface ?

4. Describe the pathlength difference between the rays of light reaching the CCD camera. Consider the thicknesses of each of these layers as being of equivalent value t.

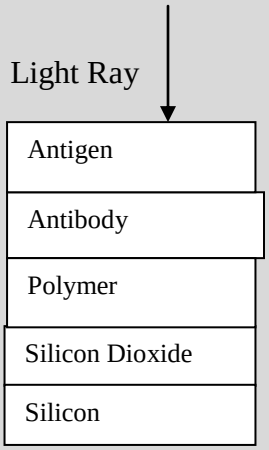
Now, a layer of antibody material is placed on the top surface. It is then cleaned so that only the antibody material which has bonded to the polymer is remaining on the slide.



5. Repeat questions 1 – 4, utilizing t as the thicknesses of the polymer, SiO_2, and silicon. Use t_{AB} as the thickness of the antibody.

6. Describe how you could use your measurements so far to derive a means of measurement for the thickness of the antibody material. Be careful to recognize in your response that the wavelength of the light passing through the slide materials is not equivalent to the wavelength of light through air.

A layer of antigen material is placed on the top surface. It is then cleaned so that only the antibgen material which has bonded to the antibody is remaining on the slide.



7. Repeat questions 5 and 6 to determine the thickness of the antigen layer, t_{AN}.

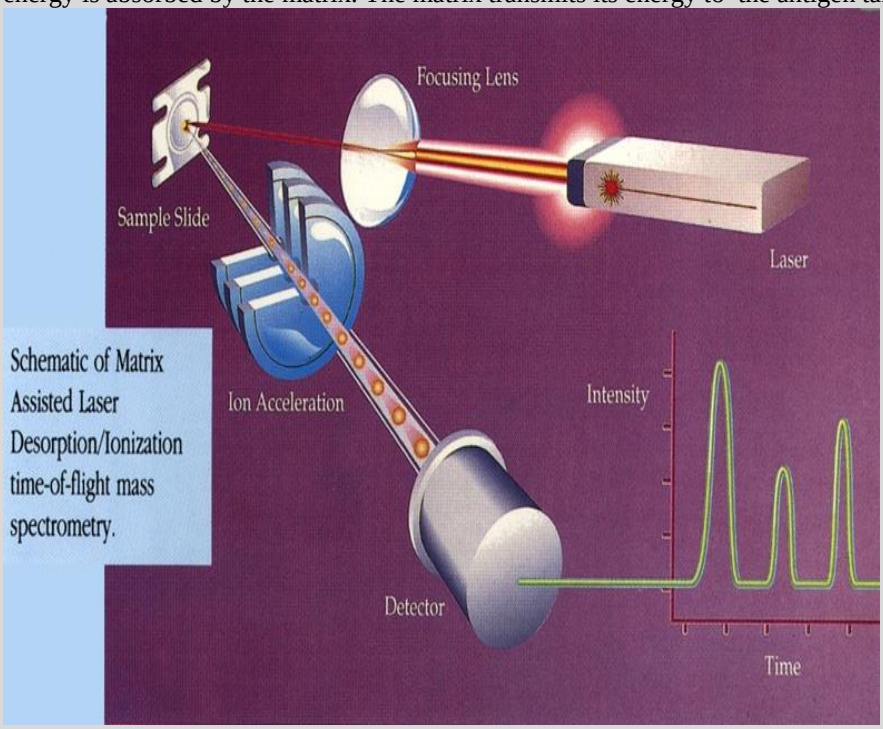
8. It is known that the deposited mass on a plate is directly related to the thickness of the layer. How would knowing the mass to depth relationship aid you in measuring the amounts of masses of the antibody and antigen materials to form the needed bonds in this reaction ?

II. MALDI-TOF Measurement

We now have a way to measure the amount of antibody which joins with an amount of antigen. Can we now determine what chemicals are present in the antibody-antigen specimen ? This would be helpful in medical analyses in which the sample being tested (from blood or urine samples) may contain other materials in high quantities. We want to assure that the chemicals tested in our IRIS sample was composed of the materials found in the proper antibodies and antigens.

To do this, we will utilize a MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight) system.

In this device, the sample that we just tested is placed onto a "matrix plate". This plate is selected so that it will absorb light of a specific wavelength. During this test, we focus laser light onto a particular spot on the chip which is to be analyzed. We then 'shoot' the laser in a series of rapid pulses. This energy is absorbed by the matrix. The matrix transmits its energy to the antigen target sample.



These pieces are ionized to their smallest possible structure and are forced down the length of the vacuum to reach a detector. This detector measures and transmits the time of flight to the computer. Using the time it took for the pieces to reach the detector and the concept of energy conservation , the mass of each piece can be calculated.

A graph will then be produced that shows the relationship between the mass per charge ratio of the pieces and the intensity of the energy of each relevant mass. Once we know this, we can identify the protein that was in the sample!

9. What is the initial form of the energy ? How would you calculate the amount of energy present ?

10. What type of energy is available as the pieces travel down the vacuum tube ? How would you use energy conservation to help you to determine the amount of mass in the moving particles ?

11. Knowing that the particles are single charged, how does knowing the mass to charge ratio for the moving particle help you in determining the identity of the moving particle ?

Explorations and Extensions **Professor Unlu's group is developing and producing a modified IRIS device which can be used by high school students to demonstrate the underpinnings of the IRIS device. If this is completed and useable by RET teachers, an actual biological experiment may be performed by students in the class.**

Lesson Development Resources **"Interferometric Reflectance Imaging Sensor", http://ultra.bu.edu/projects.asp?project=iris**

"Optical Interference Based Microarray imaging for label-free multi-analyte detection" by Ismail Emre Ozkumur as his dissertation for a Doctor of Philosophy in 2009 at Boston university College of Engineering.

Reflections

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