BOSTON UNIVERSITY

Evaluation of IRIS MALDI-TOF MS for Analyte Capture

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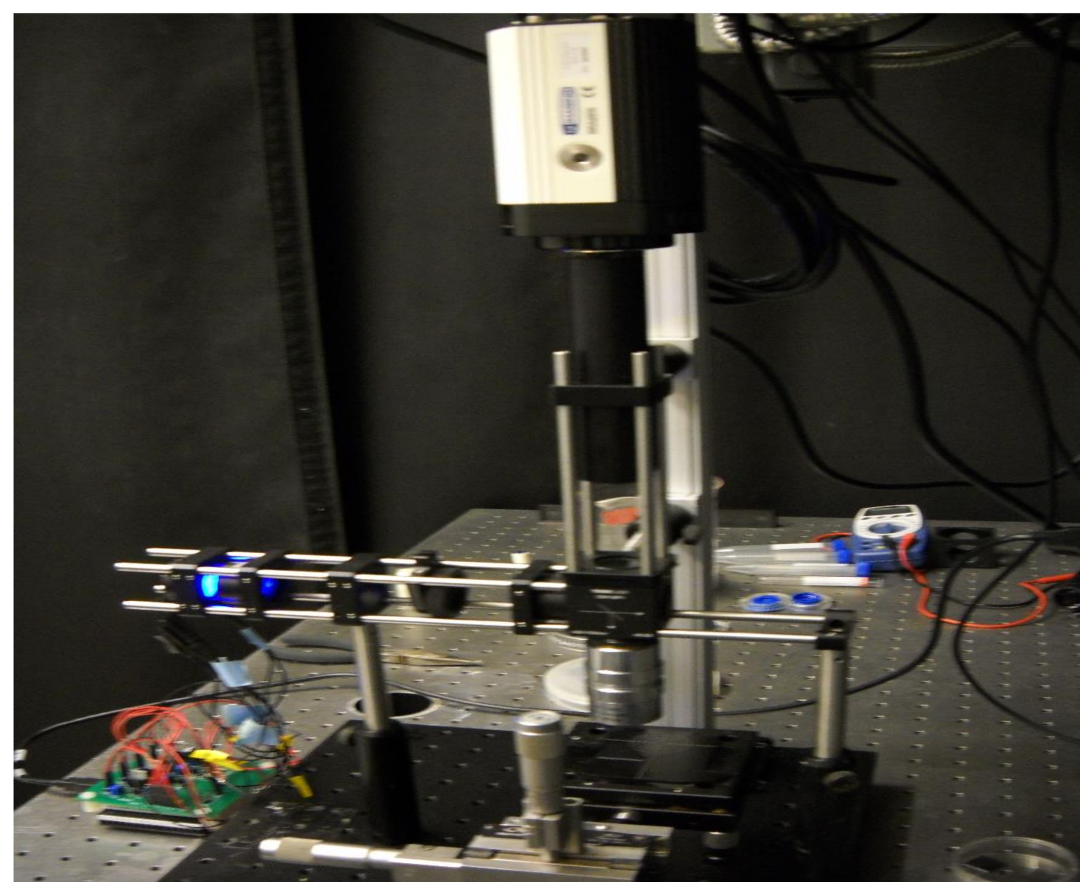
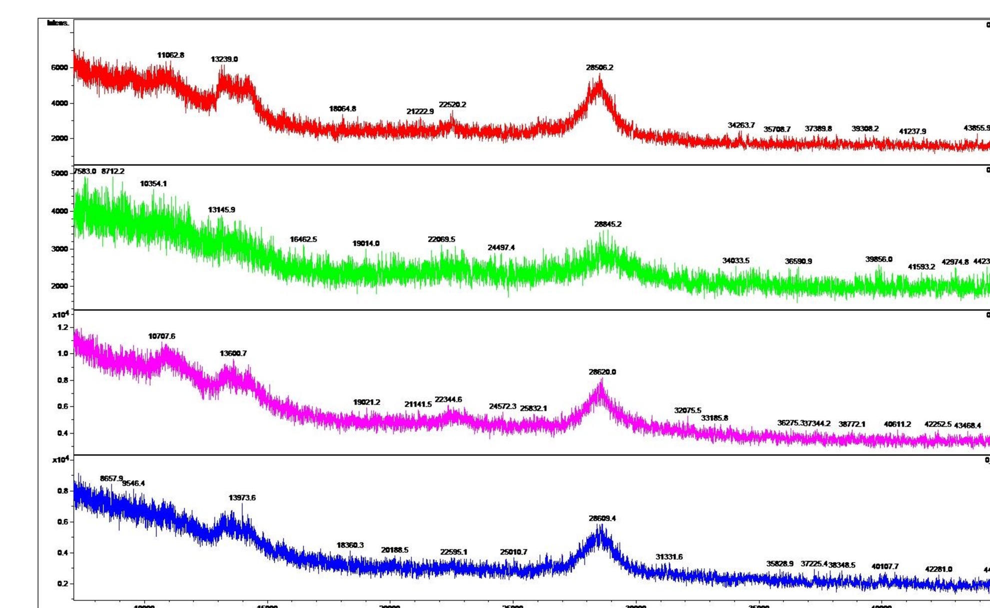


IRIS PSA Chip Results

Spot ID	Oxide (nm)	Polymer (nm)	aPSA (nm)	PSA (nm)	
a1	493.41	1.23	0.17	-0.02	polymer control
b1	493.90	1.75	-0.04	-0.02	polymer control
c1	494.21	1.79	7.03	0.04	aBI control
d1	494.83	1.60	7.69	-0.04	aBI control
AVG	494.09	1.59	1.39	0.32	Data spot averages
STD	0.56	0.34	0.47	0.15	Data spot standard deviation
CV	0.11%	21.40%	33.74%	47.60%	

Intensity vs (Mass to charge ratio) for MALDI analysis of PSA slide.



Purpose of Project

The purpose of our research is to find a better and more consistent way to measure the interaction of protein structures such as antibodies and antigens. In doing this, we are employing the Interferometric Reflectance Imaging Sensor (IRIS) to measure the mass accumulation building on our target sample and the Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) to do a mass spectroscopy of the target sample. Through the use of these systems, a quantitative measurement may be made concerning the materials interacting in the biological sample without the presence of a luminescent label.

IRIS System Operation

The IRIS utilizes a wavelength controlled light source, composed of four LEDs of different colors, to focus light onto a target being studied. The reflected light from the set of surfaces on the target is then captured by a CCD camera connected to a computer system. The computer system analyzes the interference pattern of the light reflected from the target.

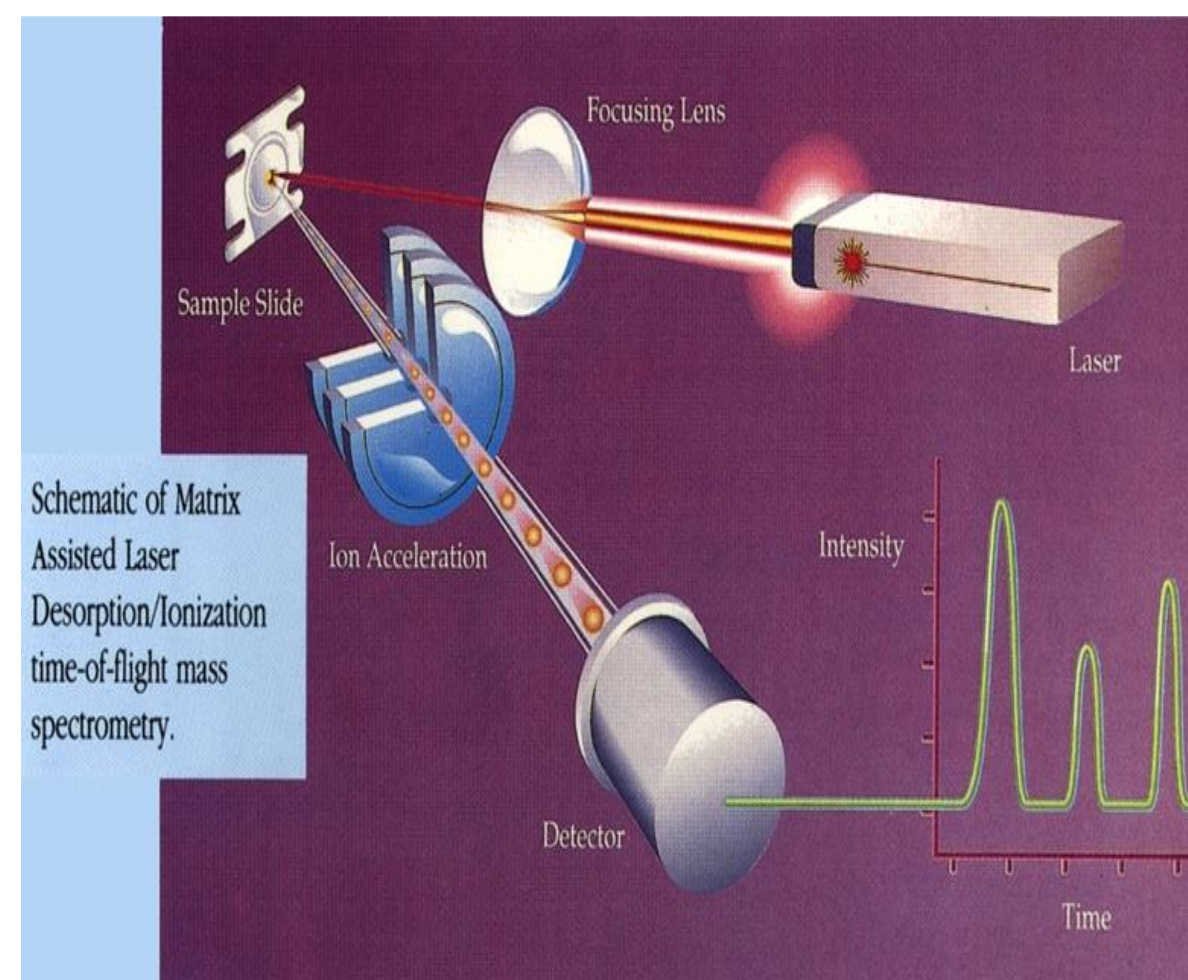
The target for our experiments is composed of a layered structure. On the base of this structure is a silicon material. Layered above this is a silicon dioxide layer. The major body of the chip is covered in HMDS. The purpose of this hydrophobic material is to help to focus the polymer and protein materials into spotted positions on the chip. The hydrophilic polymer is spotted so that the antibody will bond to it. Then, the related antigen bonds to the antibody under study.

In our study, we placed an anti-PSA antibody spot down on our polymer followed by a PSA spot. The following picture and table shows the resulting level heights of our oxide, polymer, aPSA, and PSA layers. These layers are displayed in reference to the normalized aBI control spots on the target.

MALDI-TOF System Operation

In order to determine what chemicals are present in the antibody-antigen specimen, we 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 250 to 350 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 tube to reach a detector. This detector measures and transmits the time of flight of the particle 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 is then 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!



Schematic of Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry.

Project Results

The preparation of the sample chip has proven to be the focus of our project. The nature of the problem has fallen into two categories. The placement of the polymer has proven to be inconsistent in depth and the maintenance of its mass during the testing procedure has been difficult. Furthermore, once the antibody is placed on the target, the depth of the sample is fairly high. However, when the antigen is applied and then washed, the total amount of antibody and antigen falls below the original antibody measurement. We think that this is due to the antibody layer being made up of material which is not bonded to the polymer and easily removed during the washing.

In order to overcome the difficulty in the polymer placement, we have come to utilize two experimental techniques. In preparing the sample, the polymer has been surrounded by a surface of HMDS. In this way, the hydrophobic HMDS acts to confine the polymer and proteins into a limited area on the chip. The second method deals with using the heights of the control spots as references in determining the antibody and antigen heights of the data samples.

Owing to the present chip design, we have been able to make measurements of the presence of the PSA antigen while we are using the MALDI system. In fact, as can be seen in the above graph, the spikes in the graphs of all four spot samples displayed show a clear value at approximately 28,000 daltons. This corresponds to the accepted mass to charge ratio for a singly charged PSA molecule. In addition, there are spikes in some of these graphs showing the presence of double and triply charged PSA molecules at approximately 14,000 and 9,000 daltons.

Unfortunately, the disparities in the protein sample heights on the chip have made it difficult to use the IRIS to measure the amount of the sample present.

Future Experimental Explorations :

Further effort needs to go into solving the noted issues in isolating and improving the bonding characteristics of the polymer and protein layers. This is being addressed through the redesigning of the chip configuration and the washing methods being used on the chip throughout the stages of the test procedure. Following this work, tests must be run on samples taken from more complex biological systems.

Boston University Biophotonics Research Experiences for Teachers
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