Oblique Incidence Reflectivity Difference Microscopy

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June 2006-August 2006

Introduction

Fluorescence microscopy is a technique that is currently being used in microarray analysis to determine whether or not a specific reaction has taken place. It works well with nucleic acids; however, when used to analyze reactions that occur with proteins, it results in problems because of the need to label the proteins with a molecule that will fluoresce (a fluorophore). A new, label free technique is being developed, Oblique Incidence Reflectivity Difference (OI-RD) microscopy, that does not interfere with the functionality of proteins and still allows for reaction analysis.

Background

Microarrays are powerful tools used to run multiple chemical reactions simultaneously. They are made up of small rectangular glass slides that are printed with thousands of tiny dots, each of which can potentially be a different chemical or biological compound. These slides can then be reacted with a compound and analyzed to determine what kind of reaction occurred between the compound and each of the printed dots on the microarray.

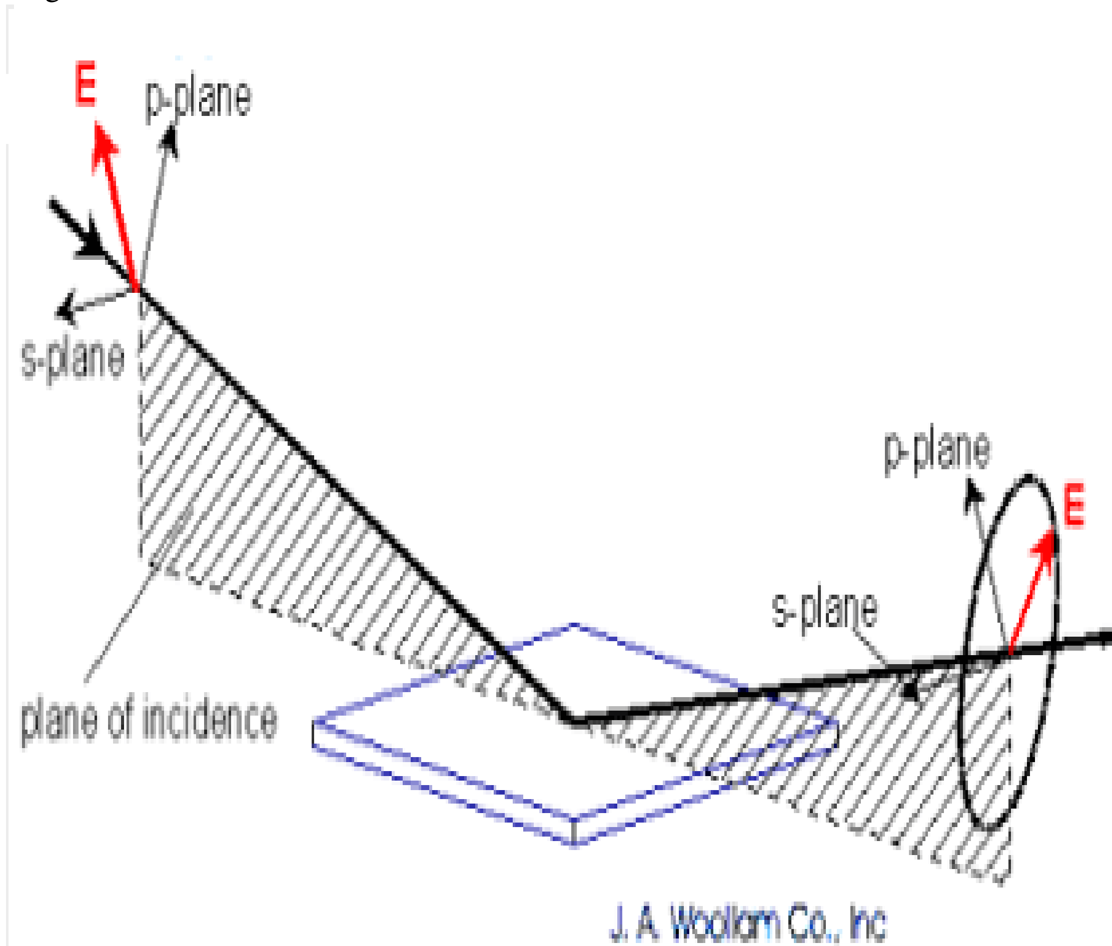
The classical technique used to determine whether or not a reaction occurred between the reaction compound and the printed chemical or biological compound is a technique called Fluorescence Microscopy. This technique involves labeling each of the printed chemical or biological compounds with a fluorophore. If a reaction occurred, the fluorophore will then glow when it is hit with a specific wavelength of light. This technique works well when the microarray dots are DNA or other nucleic acids because nucleic acids have a predictable structure and reactivity, and are minimally affected by the labeling with a fluorophore.

This technique does not work as well when the compound being tested is a protein. The reason for this is that proteins have a much more complex structure. The labeling process may disrupt the function of the protein, making it difficult or impossible to determine whether or not the protein reacted with the compound. Also, inconsistencies within the structure of different proteins results in an unpredictable efficiency in the labeling process which hinders the ability to determine the degree to which the reaction took place.

Because of this discrepancy, a new type of detection is being developed. This technique, known as Oblique Incidence Reflectivity Difference Microscopy (OI-RD) is a

label free technique. The proteins do not have to be marked with a fluorophore. Instead the reaction relies on optical ellipsometry, which is a form of detection involving the analysis of polarization states of light reflected off of a surface (in this case, a glass slide printed with microarray dots). By scanning the microarray with this technique before and after it is reacted with a compound, the presence of a reaction can be detected without the use of a labeling fluorophore by analyzing the difference between the before and after scans.

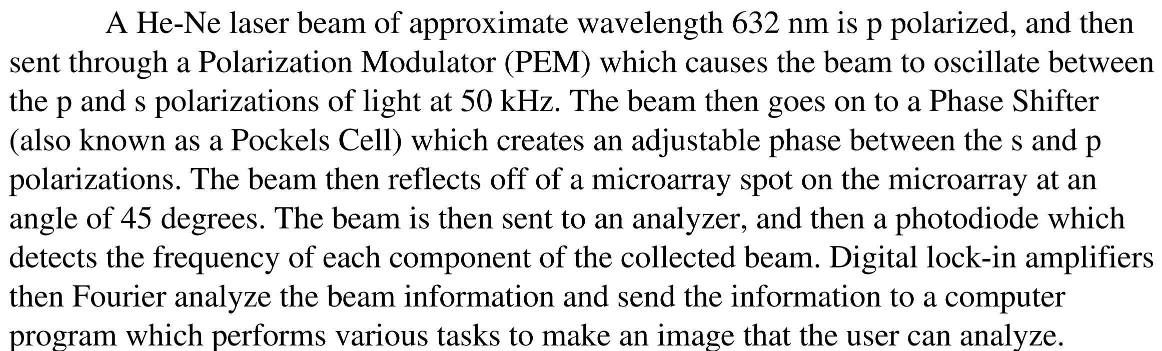
In order to conduct this type of experiment, the light beam has to be split up into 2 different polarizations, the p and s polarizations. These polarizations are illustrated in the diagram below:



The p polarized light is said to be parallel to the plane of incidence, and the s polarized light is said to be perpendicular to the plane of incidence.

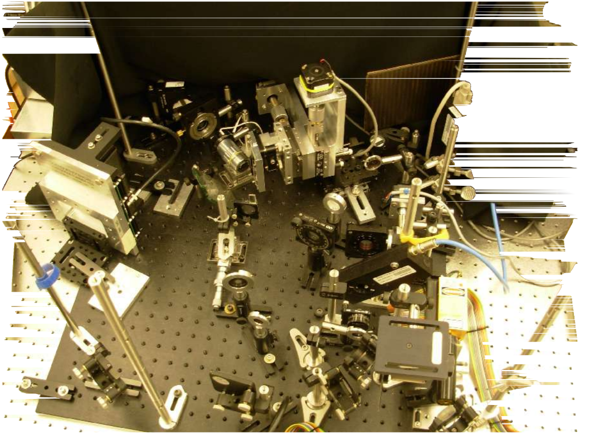
Apparatus

The microscope itself consists of several optical components put together on an optical table. The basic functionality of the components is illustrated in the following diagram:



A He-Ne laser beam of approximate wavelength 632 nm is p polarized, and then sent through a Polarization Modulator (PEM) which causes the beam to oscillate between the p and s polarizations of light at 50 kHz. The beam then goes on to a Phase Shifter (also known as a Pockels Cell) which creates an adjustable phase between the s and p polarizations. The beam then reflects off of a microarray spot on the microarray at an angle of 45 degrees. The beam is then sent to an analyzer, and then a photodiode which detects the frequency of each component of the collected beam. Digital lock-in amplifiers then Fourier analyze the beam information and send the information to a computer program which performs various tasks to make an image that the user can analyze.

The following images are taken from the actual microscopes set up in the lab:



The above pictures are of two difference versions of the same microscope, both currently under construction.

Technique

My part in the development of the new microscopes involved computer programming within Labview. We obtained two PI-motors and Mercury motor controls which have to be integrated into the microscope set up. The motors are what control the microarrays so that the laser can reflect off of each spot in the microarray.

The program I created has the ability to step and scan up and down the microarray, move acceleration of the motors, and keep track of various other things within the experiment. The change limits tab on the front panel runs an automatic program which will find the limits of where each of the motors can move to and will update them on the screen as well as implement them into the program to prevent errors in the use of the program. The following screen shots of the Labview program as it is running illustrate some of the basic functions of the program. The design and creation of the User Interface, which is what can be seen in the following pictures, was a part of the programming project as well.

Move motor | Move Both Motors | Settings | Errors | Change limits if home is reset | Data

Error: Invalid Position or Distance

STOP progam

Slow Axis

Home

Current Position
-4 µm

Maximum distance motor can move -
-92358.5 µm

Maximum distance motor can move +
65087.5 µm

Move is within limits

Maximum position to move motor to
65083 µm

Minimum position to move motor to
-92363 µm

Step

Scan then reverse

Distance to move motor each iteration
-10000 µm

Iterations
2

Number of times to repeat
1

milliseconds to wait
500 ms

Total distance motor will move
0

Distance to move motor
0 µm

Push to move motor specified distance

Position to move motor to
0 µm

Push to move motor to position

Move motor | Move Both Motors | Settings | Errors | Change limits if home is reset | Data

Error: Invalid Position or Distance

STOP progam

Total distance to move fast motor
10000 µm

Number of stops along fast axis
10

Fast Axis move within limits

Stops every
1000 µm

Maximum distance fast motor can move +
35141 µm

Maximum distance fast motor can move -
-21497 µm

Distance to move slow motor
5000 µm

Iterations 2
10

Slow Axis move within limits

Total distance slow motor will move
0 µm

Maximum distance slow motor can move +
-92358.5 µm

Maximum distance slow motor can move -
65087.5 µm

Wait time between iterations
500 ms

Step Both Motors

Move both motors to absolute positions along their respective axes

	Slow	Fast	
0	0	0	µm
0	0	0	µm
	0	0	µm

Move to Positions

Move

Slow Axis Position
-4 µm

Fast Axis Position
3 µm

Return to original position when finished
no / yes

Move both motors home
Home

Move motor | Move Both Motors | Settings | Errors | Change limits if home is reset | Data

Slow Axis

Reset to default settings

Reset

Turn motor off   Turn motor on

Define absolute position as home

0 µm

Define current position as home

DH

Caution: If you redefine Home, you should also change the limits on the change limits tab

Fast Axis Address 1   Slow Axis Address 2

Define proportional gain 35   Define Integral Limit 2000

Define Integral Gain 0   Define derivitive gain 0

Error: Invalid Position or Distance

STOP progam

Velocity 30844 µm/s

0 25000 50000 75000 100000 125000

Acceleration 2324206 µm/s^2

50 100000000 200000000 268440000

Move motor | Move Both Motors | Settings | Errors | Change limits if home is reset | Data

Error: Invalid Position or Distance

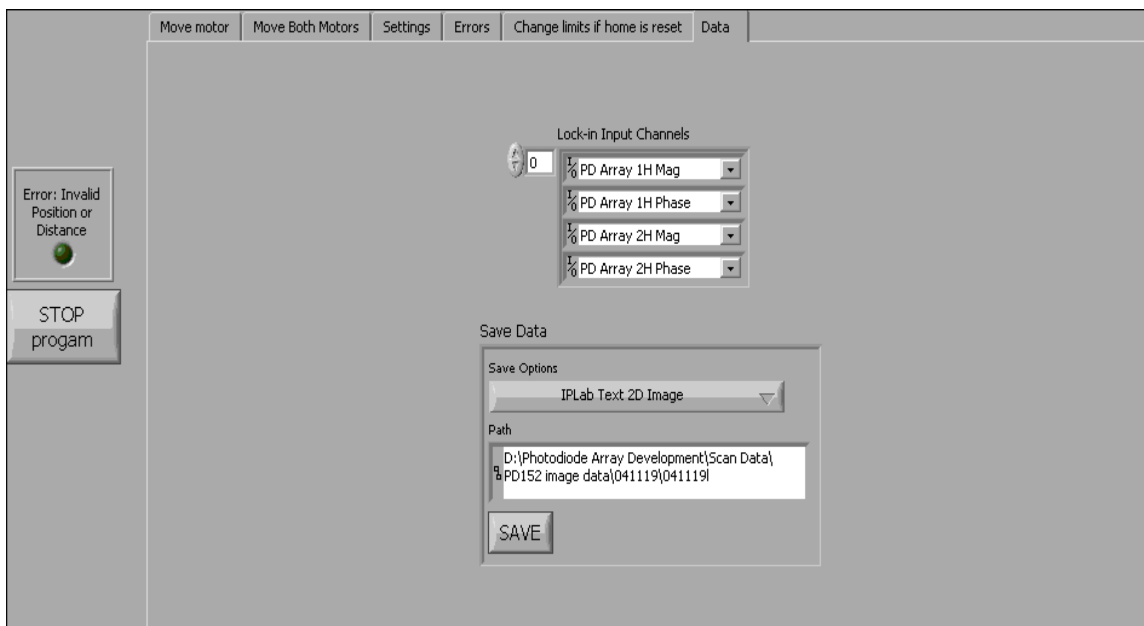
STOP progam

Limits are in µm. There are 4 motor steps per µm.

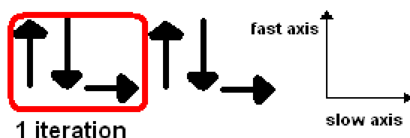
Slow Axis Min -92363 µm   Fast Axis Min -21494 µm

Slow Axis Max 65083 µm   Fast Axis Max 35144 µm

Find Limits



This diagram shows the motion of the motors when the "step both motors" button on the User Interface is pressed. One iteration is the scan up and down a "row" of the microarray (the fast motor) and one step over to the next "row" of the microarray (the slow motor)



A second part of the research done this summer was the assembly of the parts to help build a version of the microscope. The understanding of each of the components, how and why they worked within the microscope was necessary in order to complete this task. I worked alongside another REU student, Craig Wagner, and a postdoctoral researcher under the supervision of Jim Landry, a PhD candidate who is writing his research on this subject and Dr. Zhu in the assembly of this microscope.

Discussion and Conclusion

The program I have written will be used to control the motors in the newest version of the microscope that is being built. This microscope will be able to scan multiple microarrays at one time and also moves faster than the previous motors, which should hopefully decrease the time needed to scan a microarray. The summer ended before I had the opportunity to see the full use of my program and the motors, but in the future they will be used in many forms of research for the development of a useable microarray analysis tool.

In the near future, a flow cell will also be built so that reactions can be observed as they are occurring. The microarray will be suspended inside the liquid with which it is reacting with, and the scan will be continuous to provide data on the speed as well as the

success of the reaction. Previous techniques do not allow the analysis of the reaction as it is occurring; only the detection of the reaction after it has taken place. The lab has also recently purchased a microarray printer, so that the microarrays can be printed within the lab. The researchers in the lab will be working in conjunction with researches at the University of California Davis medical school for future development and integration of the microscope into research.

Some applications for this microscope could be in the development of new drugs for pharmaceutical companies. Each microarray can contain thousands of different compounds, which essentially means that thousands of reactions could be conducted simultaneously, and the best candidate for further research and development could quickly be determined. The flow cell construction would be a further benefit in this area, because the relative time needed for the reaction to occur could be included in the analysis. This technique could also be used to detect the presence of certain compounds within a sample.