Instrumentation Report: Creation of a Scanning Laser Microscope

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Abstract

The aim of the project isour project was to develop a Scanning Laser Microscope (SLM) using a cost efficient method for use in both biological and material research. The microscope sends a laser beam through multiple lenses and an aperture to focus the beam at a single point on the sample. The location of the point on the sample is determined by the angle of a mirror, which is controlled by two stepper motors. Some amount of the laser is reflected off the sample towards a photodiode, which measures the intensity of the reflected laser. The laser will scan across the surface of the sample as the synchronized photodiode reads data from the laser. We plotted the data into a square matrix, creating an image of the sample. We mounted a sample grid and found the calibration factor of 12.3 steps of the stepper motor corresponding to one micron across the sample and an estimated the spatial resolution of our microscope to be 1.3 microns. We then used our SLM to image an integrated circuit chip and crushed salt. The SLM we built is capable of creating images with a resolution high enough to distinguish features on the chip. Our SLM will be a useful research tool for imaging both biological and fabricated materials.

I. Introduction

The devised scanning laser microscope (SLM) allows the experimenter to scan a two dimensional plane of the sample by moving the focus of an incident laser beam along the sample, recording voltage values with a single-pixel photodiode. This is in contrast to a conventional microscope which uses an area detector to take in incoherent, white light reflected off a sample [2]. The quality of the image coming from our microscope relies on the characteristics of the incident beam (e.g. wavelength, area and intensity) [6] and the aperture of our objective lens [1]. By focusing the laser light onto a very tiny point we are theoretically limited in lateral resolution only by the diffraction limit of the incident light. However, achieving resolution of this quality is very difficult experimentally due to aberrations, imperfections and the influence of noise. This setup allows us to move from point to point by moving a small mirror, controlled by a LabView Virtual Instrument (VI). The setup is such that the lateral focal point on the sample is moved by an angle change of the incident light–this is done without walking the beam [2]. The simplicity of this scanning setup and technique allows wide accessibility among many levels of laboratory experience.

It is also possible to modify this setup to increase its usefulness in a variety of lab settings. The microscope may be modified with a confocal pinhole [5]. Through a process of running multiple scans of various depth, the sample may be imaged along the optic axis to create an image of the structure, which may be very useful for sampling thick biological tissue [3]. There are a variety of different modifications the user may implement to pursue different goals. The ability to modify this fundamental setup to increase usefulness in biological and other settings means this design will continue to be pertinent far into the future.

II. Principles of Operation

The principle of the SLM, in theory, is very simple. A laser is reflected off a specific point on the sample and the intensity of that reflected light is picked up by a photodiode. If the sample is more transparent, less light will reflect back while if the sample is less transparent, there will be more light bouncing back; using this data, we can create an image of the sample.

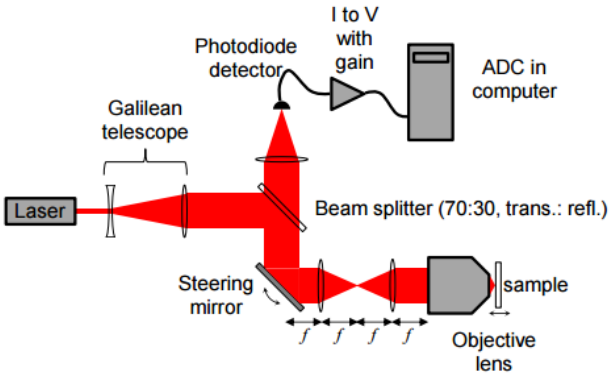


Fig. 1: This shows the skeleton of the microscope, including the laser and the sample. The motors change the angle of the mirror, which changes the location of the laser on the sample. The laser reflected off of the sample then bounces back to the photodiode, where the analog data is collected.

As shown in Fig. 1, however, the laser system is slightly more complicated. A laser is emitted from a source and goes through a Galilean set of lenses which widen the beam. It then goes through a beam splitter, where some of the beam goes straight and the rest of the beam refracts ninety degrees toward the mirror. The beam reflects off the mirror which is controlled in vertical and horizontal angle to the stepper motors. Since the angle of the mirror is controlled by the stepper motors, the location of the reflected beam is then controlled allowing the user to scan the beam across the surface of the sample.

Then, the light goes through another set of lenses that reposition the beam so that it hit the sample directly. Lastly, the beam then goes through an apparatus that will change the position of the focus on the sample based on the angle of the inputted beam without changing the focal distance of the beam. At this point, the laser will hit the sample and some amount of it will reflect (determined by the reflectivity

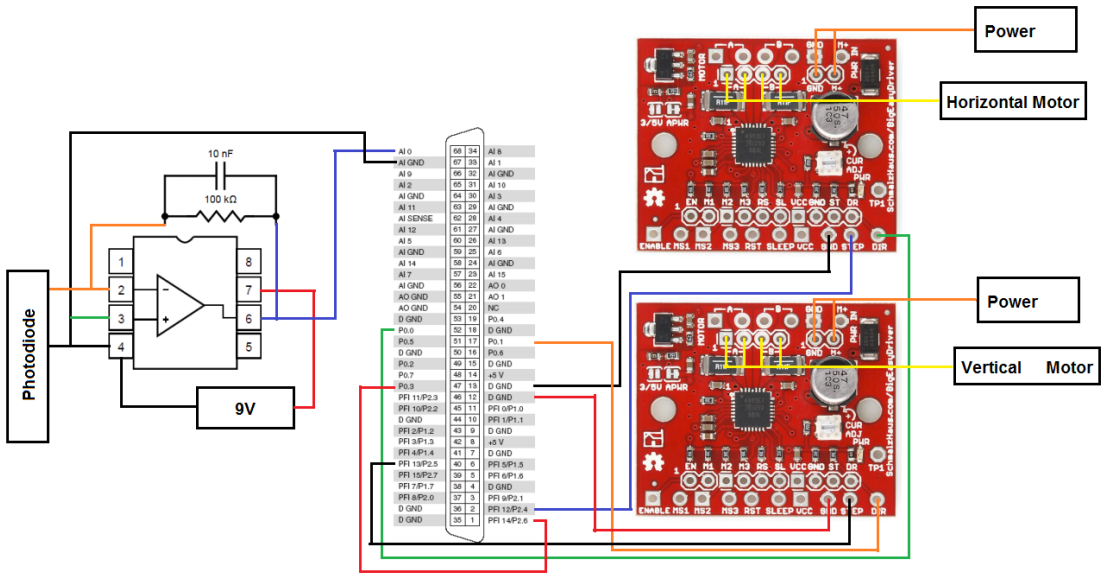


Fig. 2: Here is the wiring setup for our microscope. In the center is the DAQ board with the labeled ports. The computer is able to read and write from these ports. Six of the ports connect to the stepper motor drivers, which are pictured to the right of the diagram. [2] These stepper motor drivers then connect to the actual stepper motors and to a power supply as indicated. The DAQ board also connects to our photodiode circuit, pictured on the left. The photodiode circuit uses an Operational Amplifier (Op-Amp) increase the signal and make it easier for the computer to recognize the changes in the signal.

of the point on the sample). The beam will return through its path and some of the beam will go to the photodiode, which detects the signal.

In order for the microscope to work correctly, we need to be able to control the movement of the mirror and detect the reflected beam using the same clock in order to put the data together correctly; for this we have to use multiple subsystems.

A. *Motorized mirror control system*

1) Hardware Setup: The motors are driven by two Easy-Drivers. These easily controllable stepper drivers each take DC power in, converted from mains by a small AC to DC converter. The two drivers each connect to the Data Acquisition board (DAQ Board) in three places: one wire connects to ground, one wire receives a signal to indicate direction for that motor; one wire receives an alternating signal which communicates the speed and total step of the motor. This is shown in Fig. 2. The drivers take the signal from our VI through the DAQ board and then send the appropriate voltage pulses to each motor to turn them as indicated in LabView.

2) Software Development: For the software portion of the stepper motors, we created a VI to control them. First, we created user inputs from the front panel that allowed the user to name which motor should move, which direction the motor should move, the frequency of the movement, and the number of steps (or number of fraction of rotations) should be used. Based on which motor was selected, a case structure is used to determine which port of the DAQ board the pulse train and direction should be sent to. Then, the program uses the number of steps and frequency to determine the duration of the pulses sent, the amount of time the motor should run, and the number of samples. A DAQ task was created and then started which wrote a zero or a one to the port for direction based on the user's input. Once this task starts, the program then creates another task, which is the pulse train, and uses a hardware timed clock to send out this pulse train to the selected port on the DAQ, causing the stepper motors to move. Once the pulse train is completed, the direction task will also complete and any errors will be outputted to the front panel of the VI. We then made this VI into a subVI, so it could be placed in the VI to control the entire microscope; the user inputs will be wired into the subVI so we may run our motors while collecting data.

3) Testing Motors: After assembling both the physical setup and the program to run the motors, we sent pulses to the motors with varying frequencies to test the limits of the motors. Overall, we found that the highest frequency of the pulse train we could send to the motors was about 17,000 Hz. Frequencies higher than this would cause the electromagnets to malfunction, resulting in the motors no longer spinning. This limit is more than acceptable for our purposes since scanning at this high of a rate will result in

many inaccuracies, so this limitation is very practical.

We faced only one issue in this step, which was that the motors we worked with have relatively low torque and slipped if met with some resistance. However, we were able to fix this issue by replacing the tubes joining the motors to the mirror. We encountered only minor slippage in the final stages of the project.

B. Signal Detection

The photodiode detection circuit is the means by which signals from the laser are recorded. Broadly, the light that is reflected off the sample is detected by the photodiode, which sends electrical signals to the DAQ, then read by an Analog to Digital Conversion (ADC) Labview VI.

Light reflected off the sample travels through the apparatus housing, through the beam splitter, and ultimately focused on the photodiode detector. As seen in the wiring diagram (See Fig. 1), the photodiode then sends a voltage to the circuit on the protoboard, which connects the photodiode to the Op Amp. The Op Amp, using a 9V battery and a resistor, amplifies the signal received by the photodiode. The amplified signal is sent to the DAQ so the computer is able to access the signal. The ADC Labview VI reads the analog signal, converts it to a series of 0's and 1's, and displays the recorded light levels (over a certain period of time) on a waveform graph.

The range of detectable signals by the photodiode was on the order of -1V to 1V, with most laser detections at approximately 0V to 0.4V. The recorded voltage depended on what signals are sent to the photodiode and the sources of noise present. However, the noise we saw is constant throughout the measurements, so the differences between the reflected light while scanning gave an image of the sample, so the noise is not much of a concern in this scenario. Ultimately, this photodiode detection circuit can detect a wide range of signals and was essential to the construction of the microscope.

C. Scanning and synchronization

In order to get both of the stepper motors and photodiode to run at the same time, they need to use the same hardware timed clock. To do this, we outputted the a pulse train to the DAQ board. The stepper motors used this hardware timed signal to run, while the photodiode read the hardware-timed clock and used it as a software-timed clock.

The method to scan that we used is as follows: the program takes in the point for the starting corner, which the beam is moved to. The size of the part of the sample that the user wants to sample is also inputted so the microscope knows how large the region to scan is. The microscope moves the laser in a row across the sample taking a data point at a regular interval as it scans (the user inputs how often they wish points to be taken). At the end of each row, the laser is moved down to the next row, back to the beginning side of the sample, and this row is then scanned across. The data points that are taken in are put into a square matrix that represent the sample. With the values of this matrix, we created a figure with different color intensities depending on the value of intensity at every point to create an image of our sample.

In order to create images as described, we scanned various samples, each of varying shapes and sizes. This required trial and error to find the correct mounting and positioning needed to get a clear, interpretable image with the correct scale. Once the setup was calibrated and focused we were able to get high resolution images of many samples.

III. Preparation of samples, calibration, and spatial resolution

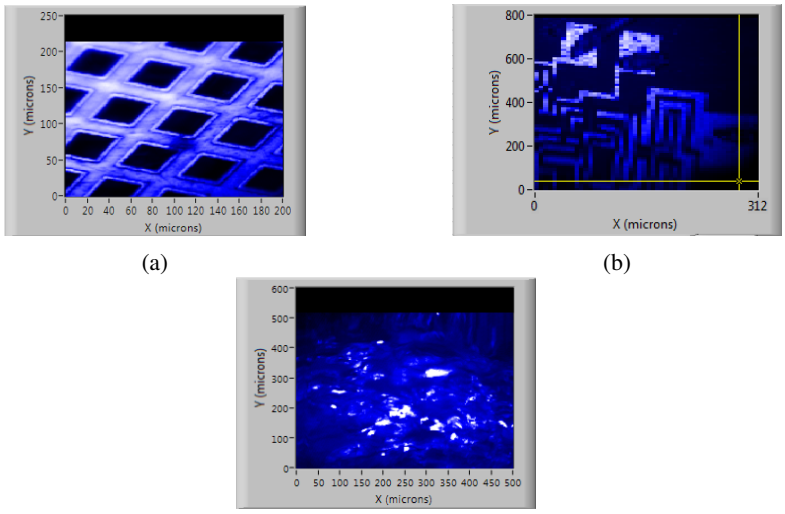
A. Sample mounting and positioning

In our SLM, samples needed to be placed perpendicular to the incident laser in the microscope after the aperture such that the laser could scan across them. In order to mount the samples, we used an adhesive such as double sided tape to place a sample in the middle of a circular slide that fits into our microscope. We then placed the slide into the microscope and then checked to see that the laser was focused on the sample. If the laser was not focused on the sample, we then tried to either move the sample itself into the beam's path, or readjust the starting point of the laser. Once we have completed this, we imaged the sample and adjusted the X and Y coordinates of the starting point to find the correct spot on the sample that we wanted to image.

B. Calibration and spatial resolution

The calibration factor of our microscope is a multiplier that determines the number of steps the stepper motors will take in order to move the laser one micron on the sample. To find this value, we started by setting the calibration to one and imaging a grid that we knew the pitch to be 62 μm. Using this image and the LabView software, we found the X and Y coordinates of the beginning and end of one side of the grid. We then used the difference between the two points to calculate the pitch of the grid. Since we know this value should be 62 μm, we found the ratio of the imaged pitch to the correct pitch to determine the calibration factor. When we computed this, we determined the calibration factor to be 12.3 steps per μm. We put this value into the microscope settings, scanned the same grid, repeated the calculation to find the pitch and calculated 62 μm, so we confirmed our calibration factor is correct.

To find the spatial resolution, we started by imaging with a low number of points per line, so there was a relatively large space between each reading by the photodiode. We then incremented this value slowly and looked at a singular line scan over the same area. When the distance between the points became low enough that the line scan looks identical to a slightly higher points per line, we have reached the resolution limit and the microscope cannot discern details smaller than this spacing. We found that reading one point per 16 steps of the motor is the resolution limit. There was more detail in this graph than the graph that scanned one point every 15 steps, but was identical to the graph scanning one point every 17 steps. We divided the 16 step resolution



(a) (b)

(c)

Fig. 3: Pictured above are three different samples imaged using the SLM microscope. Image (a) displays a TEM grid that was used to calibrate the microscope. Image (b) is an Integrated Circuit Chip. In this image we are able to see connections that are too small for the naked eye to discern, making it an interesting sample to be able to study. Pictured in (c) is an SLM image of crushed salt. We are able to see very fine NaCl crystals using the microscope, which shows how well we can see tiny minerals.

limit by the calibration factor to get a spatial resolution of 1.3 μm.

With all subsystems operating properly, and the calibration details calculated, we mounted several samples to the microscope. We imaged a TEM grid, Integrated Circuit Chip, and crushed salt crystals.

IV. Example Measurements of Several Samples

Figure 3a shows an image of a TEM grid taken from our SLM. This grid was used to calibrate our microscope since we know its pitch to be 62 μm. The grid does appear skewed, so this could potentially cause some error in our calibration factor. The second image, in Figure 3b, displays an Integrated Computer Chip. In the image, you can see two solder pads from the manufacturing of the chip, as well as some of the connections in the chip. The final image, found in Figure 3c, shows crystals of NaCl, commonly known as salt. We crushed the salt and mounted it on the microscope. We are able to distinguish crystals as small as a few microns, which highlights how detailed the SLM images can be.

The images we produced give plenty of insight to the possible implications of scanning laser microscopes. For example, one of the practical uses of our microscope would be to see if there were any errors in the chip since we would be able to detect a breakage in the connection. The importance of the SLM we created, however, extends beyond its imaging capabilities.

V. Conclusion

By constructing a SLM, we were able to create a cost efficient method of scanning materials that has a reasonably good resolution. This microscope is easily controllable and can be fixed quickly as the parts are interchangeable.

This microscope is capable of scanning a samples on the scale of microns and producing an intuitive image for the user. It can image with a spatial resolution of 1.3 μm, which is relatively close to the optimal diffraction limited resolution.

The microscope we have created can be implemented in a variety of disciplines to generate the desired results. With the ability to distinguish between different connections on a circuit chip, our microscope shows potential to be used practically in the future.

References

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