ENSC 476/895
Biophotonics
Lab 1 - Confocal

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Purpose

The reason we are doing this lab is to learn about confocal imaging. During this lab we should be able to compare our experimental measurements with the expected values of measurements. We will choose two optics from the many we were given and we will use our knowledge of confocal imaging to be able to focus the provided sample.

Methods

The laser, beam splitter, confocal aperture, and galvos were already set up by the Teaching Assistant (TA). Our responsibility was to align the optical relay lenses, objective lenses, and the sample. Figure 1 below shows the schematic of what our optics setup is. We are able to see the data from our results through a multifunction data acquisition board (DAQmx board; National Instruments Inc., PCI 6251) which is connected to a computer.

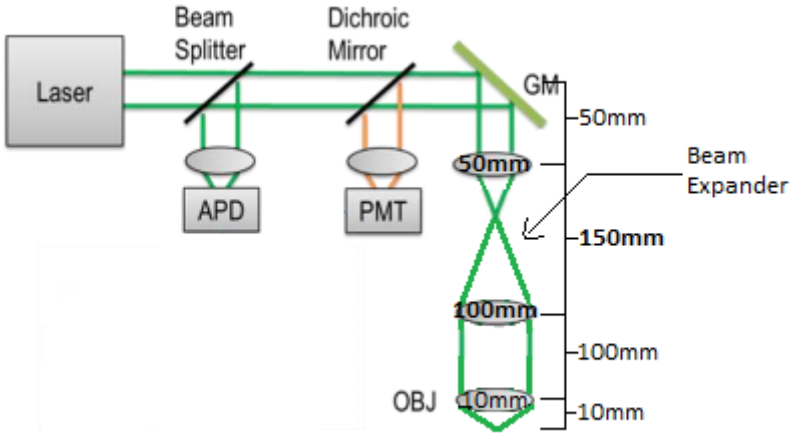

Figure 1: Optics setup schematic

Through beam scanning optics design we knew that we had to choose two lenses. These two lenses were used to design a lens configuration between the scanning mirror and the objective lens. This enables the focused beam to scan the sample provided. The TA told us that the focal point of the objective lens was 10mm (1cm) and the two lenses we chose had focal points of 50mm (5cm) and 100mm (10cm). Note: The lenses were labeled in mm, but to measure the lengths between the lenses we only had a ruler which could only measure in cm.

Starting from the scanning mirror we aligned the 50mm lens at a distance of 50mm. Then aligned after the 50mm lens at a measured distance of 150mm away was the 100mm lens. This distance was chosen because it was the combined focal length of the two lenses allowing the beam to focus at 50mm and then be collimated after another 100mm. The 10mm objective lens was aligned 100mm away from the 100mm lens. With this setup the sample was then placed 10mm away from the objective lens because this is the focal length of the objective lens. With the sample at 10mm away from the objective lens the sample can be focused.

Results

A CyrstaLaser LC Diode Pumped Green 532nm CrystaLaser was used in this experiment. Therefore we know that the wavelength of the laser beam is 532nm and the initial waist is 0.36mm. Then we get our estimated focal spot size to be **4706.3nm**:

$$Focal\ Spot\ Size(W_f) = \frac{Wavelength * Focal\ lenght}{\pi * Inital\ waist}$$

$$Focal\ Spot\ Size(W_f) = \frac{532nm * 10mm}{3.14 * 0.36mm}$$

$$Focal\ Spot\ Size(W_f) = 4706.3nm$$

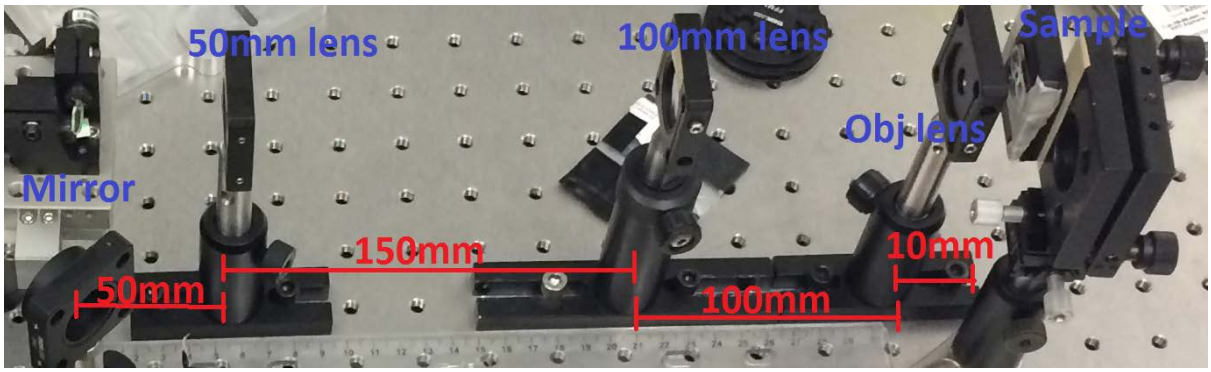

Figure 2: Aligned setup of the optical relay lenses, objective lens, and the sample

Figure 2 above shows the results of our aligned lenses. On the bottom of Figure 2 shows the ruler used to measure the distances of the lenses. There should be a little +/- differences from the exact values of the distances because we have to guess exactly where the middle of the lenses are.

Figure 3: Image retrieved from the setup configured in figure 2

Above is the image from the scattering sample we were provided by the TA. The letter ‘E’ can clearly be seen from this acquired image therefore our experiment was a success since

the image was properly focused at the distance we calculated the lenses and sample should be.

We then replaced the scattering sample with a resolution target provided by the TA. Figure 4 and Figure 5 below shows the result of our setup after replacing the scattering sample with the resolution target.

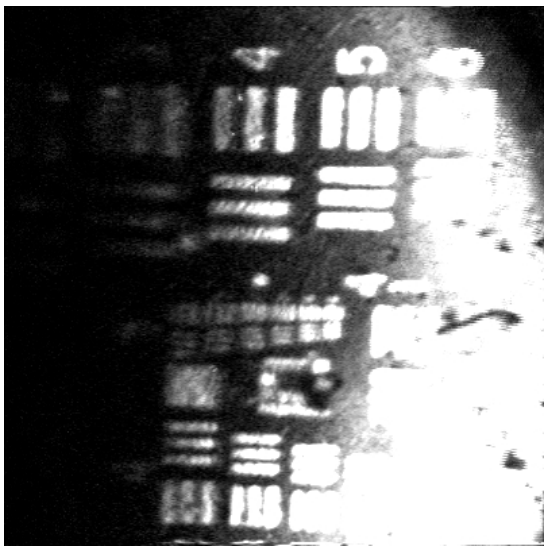

Figure 4: Acquired resolution target with a blurry group 5

These two figures are similar but we are presenting both because on Figure 4 the left and middle part is blurry while on Figure 5 only the right part is blurry. If we only used Figure 4 then we would have concluded that group 4 was our maximum resolution.

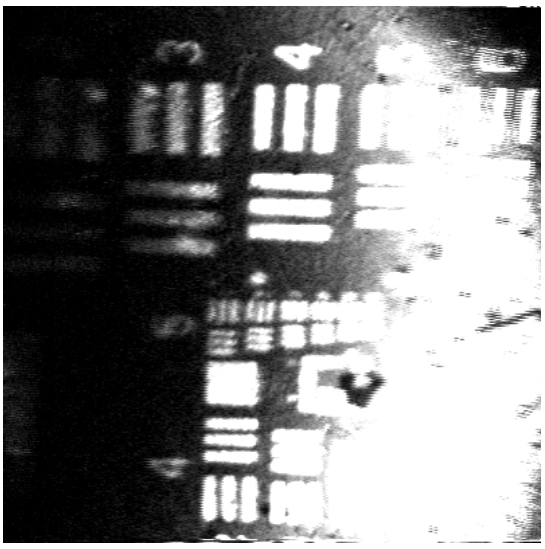

Figure 5: Acquired resolution target with a sharper group 5

However, Figure 5 shows that our resolution is actually better. We can clearly see from Figure 5 that we can see at least as resolute as possible group 5 at element 2. With the acquired resolution target from Figure 5 above we can then estimate our actual resolution calculated below which we found to be **35.9 line-pairs/millimeter**.

$$Resolution = 2(Group\ Number + \frac{Element\ Number - 1}{6})$$

$$Resolution = 2(5 + \frac{2 - 1}{6})$$

$$Resolution = 35.9 lp/mm$$

We had some time left in the lab so we also did the fluorescent sample. Figure 6 below shows our results after focusing the new given sample.

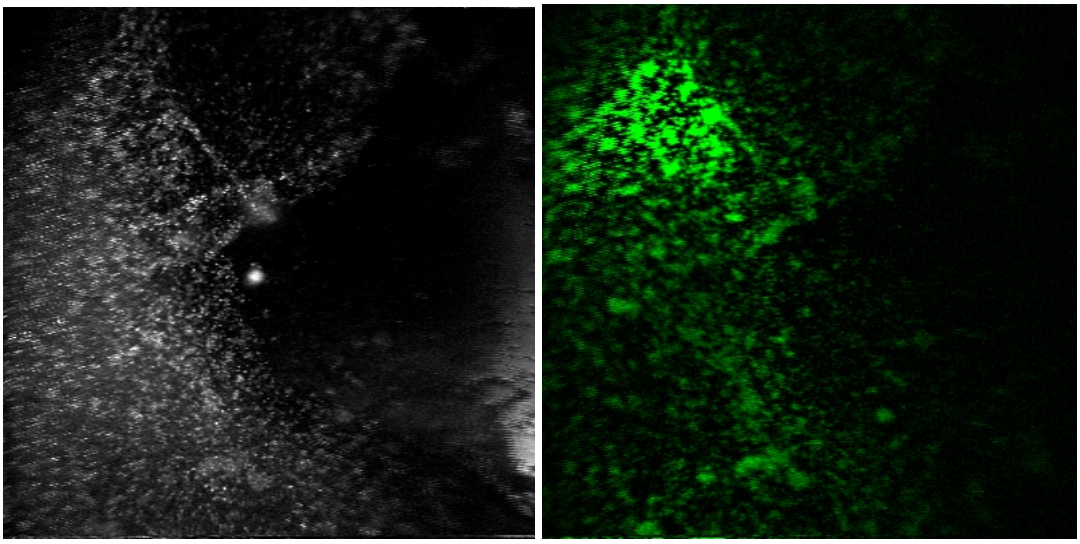

Figure 6: Acquired image from the fluorescent sample

The TA told us to turn off the lights and then he turned on the power supply and cranked it up to 6 Volts. At this voltage we were able to retrieve the image from Figure 6 above. We knew that our results were good because the fluorescent image on the right (the green image of Figure 6) resembles the left image. There are also some parts (like the right side) on the left image that are not seen on the right image.

Discussion

At first we thought that the distance from the mirror to the 50mm lens did not matter because that beam is just a collimated beam therefore it would not have mattered how far the 50mm lens was from the mirror. However, after setting up the rest of the lenses and the sample as seen above in figure 2 the data we received that we could see on the computer screen was a black color and some light reflections. We fixed this problem by following the diagram seen in Figure 1 and Figure 2.

Another issue we encountered was when we properly aligned the lenses and the sample we still could not acquire an image. This was easily fixed by centering the laser beam as much as possible on the lenses. At first we did not get any images because the lenses were either too low or too high which angled the laser beam improperly which did not focus the image.

Conclusion

This lab helped us to analyze how the beam expander is useful for laser application. We learned how to calculate the spot size of the laser as it travels from the source through the beam expander. Most importantly, we learned how to place different lenses in order to get the desired beam expander. We also noticed the relation of intensity with the laser's spot size and the laser scanning. As a whole, it was a good experience of our first touch on handling laser and other optical instruments to study confocal imaging.