Large Infrared Optical Tweezer Array

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Abstract

Infrared Optical Tweezer Array

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A summary of an attempt to build a large optical tweezer array for trapping cancer cell nuclei is described. Our system was ultimately unable to trap a large array of cell nuclei but was able to trap a large array of polystyrene beads. Our optical trap array method is described as well as its drawbacks and advantages and the potential modifications that could improve the systems performance. The system utilized an amplitude-modulated spatial light modulator to generate an array of individually addressable optical traps. The optical source for trapping radiation was a neodymium-doped yttrium vanadate crystal laser (Nd:YVO₄) which produces light at a wavelength of 1064 nm. (The system utilized a Texas Instruments digital micro-mirror device as a spatial light modulator, a Zeiss Axio-Imager microscope to view the sample and focus the laser light, and a Spectra-Physics CW, Nd:YVO₄, 1064 nm laser.)
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Chapter 1  Introduction and Motivation

1.1 Introduction

Methods to manipulate the position and orientation of submicron particles nondestructively are incredibly useful tools for basic biological research.\(^1\) Perhaps the most widely used physical force to achieve noninvasive manipulation of small particles has been dielectrophoresis (DEP).\(^2\) However, DEP on its own lacks the versatility and precision that are desired when manipulating cells since it is traditionally done with stationary electrodes. Optical tweezers, which utilize photon radiation pressure to exert forces on small particles, achieve this desired versatility and precision.\(^3\) However, a major drawback of this approach is the high radiation intensity required to achieve the necessary force to trap a particle which can damage biological samples.\(^4\) A solution that allows trapping and sorting with lower optical intensities are optoelectronic tweezers (OET) but OET’s have limitations with fine manipulation of small particles; being DEP-based technology also puts constraint on the properties of the solution.\(^5,6\)

There are two methods that have been utilized in our lab to decrease the intensity of the radiation and achieve orientation control of small particles. The first method is plasmonic tweezers which use a random gold nanoparticle (AuNP) array as a substrate for the sample. The AuNP array converts the incident photons to localized surface plasmons (LSP) which consist of resonant dipole moments that radiate and generate a patterned radiation field with a large gradient in the cell solution.\(^7\) The plasmonic approach allows for fine orientation control of ellipsoidal particles and cells with low optical intensities because of more efficient optical energy conversion into mechanical energy and a dipole-dependent radiation field. One main problem with plasmonic tweezers is that the LSP’s generate a considerable amount of heat. This heat generates convective flows and thermophoresis which can be powerful enough to expel submicron particles from the trap.\(^8,9\)

The second approach is photonic crystal tweezers which make use of photonic crystal structures to scatter incident light very efficiently into diffraction modes.\(^10\) Ideally, one would make this structure out of a dielectric material to avoid the
same heating problems experienced with the plasmonic tweezers but in our labs approach an aluminum diffraction grating was used as a one-dimensional photonic crystal substrate. Though it is not a semiconductor it did not experience significant heating and effectively trapped small particles. Alignment of particles with the grating substrate conceptually validates the proposition that a 2-D photonic crystal could allow precise rotation of non-spherical micron sized particles.\textsuperscript{10}

This thesis describes the beginning stages of development of a large array of such nanostructure enhanced laser tweezers (NELT). Because development of the nanostructure substrate for our optical tweezers was examined extensively by my predecessor, Ben Wilson, my task was to develop the optical system to guide light to the sample of particles in a way that effectively achieved an array of trapping laser spots. The final goal of this effort is the fabrication of a large NELT array that is capable of trapping ovarian cancer cell nuclei so that they may be studied in a more efficient manner.

1.2 Motivation and Achievements

The final goal of this research was to generate a large array of nanostructure enhanced laser tweezers (NELT) with individually addressable traps. This trap array was to be used by our collaborators to capture and image purified ovarian cancer nuclei immunostained for transcription factors signifying activation of the PI3K-AKT oncogenic pathway. Visualization of this pathway would allow our collaborators to determine the heterogeneity of PI3K-AKT pathway activation within tumor sites and across stages and locations of ovarian cancer progression in individual patients. The accomplishment in this MS thesis research was to revise our previous NELT system that uses a HeNe laser to a system that uses an Nd:YVO\textsubscript{4} laser with incorporation of TI’s digital micro-mirror device (DMD) spatial light modulator. Trapping of a small array of polystyrene beads was demonstrated. Trapping of a single nucleus was also demonstrated using the new laser.

Chapter 2 Prior Work

2.1 Trapping of Beads and Biological Specimen

There are many different approaches to optical trapping of single and multiple particles. The most common approach to trap
a single particle is to utilize a microscope to focus a laser beam down to an appropriately sized spot. The microscope is composed of a light source which illuminates the sample, a beam splitter that directs the light from the source to the sample and also allows a camera to view the sample and a microscope objective that directs light to the sample and magnifies it for viewing. In order to turn the microscope into an optical tweezer, the optical path requires a specific set of optical filters and polychroic mirrors which allow the user to view the sample while the laser is directed through the objective and focused onto the sample. If these filters and mirrors are not used, saturation and damage of the camera can occur as well as damage to the eyes of anyone looking through the eyepiece. An optical trap additionally requires a set of optics external to the microscope in order to steer the beam into alignment with the optical axis of the microscope.

Prior to my arrival at the University of Washington, Xiaoyu Miao and Ben Wilson had performed optical trapping in Professor Lih Lin’s photonics lab at the University of Washington. The individual optical trap system he had used turned into a simpler model for our new system designed to trap an array of particles. The prior system utilized a HeNe laser capable of producing 35 mW of power at a 632.8 nm wavelength. The laser was redirected off of steering mirrors into the microscope where it reflected off of two dichroic mirrors before focusing down to the sample through the microscope objective. A drawing of the system is shown below:
Chapter 3    Our Approach

3.1 Creating an Optical Trap Array

The basic optical tweezer is constructed of a laser, beam steering mirrors, and a microscope with a set of beamsplitters that direct the different wavelengths of light to where they need to be. The elements for the basic optical tweezer set up are essentially the same in our new set up as they were in the optical tweezers that Xiaoyu and Ben had built. The changes that we made were to use a new neodymium yttrium vanadate (Nd:YVO₄) laser capable of producing over 5 watts of CW 1064nm power to provide enough power to each individual trap. This new wavelength required a new custom set of polychroic mirrors to be used, as well.

Optical tweezer arrays require additional elements in the system that allows the laser to be split into multiple beams which generate separate optical traps. The element that actually provides this separation is called a spatial light modulator (SLM). Different types of SLM’s utilize phase modulation or amplitude modulation of coherent laser radiation to produce separate trapping spots at the sample. In addition to the SLM, beam expanders are required to enlarge the typically small laser beam into a size that can effectively be modulated.
3.2 Spatial Light Modulator

The choice for what spatial light modulator (SLM) to use was a very important one. After reviewing many different SLM’s, we decided to attempt the trap with an amplitude modulated digital micro-mirror device (DMD) from Texas Instruments. This Digital Light Processing (DLP) technology is made up of a silicon chip with a large array of independent mirrors on it. The chip’s micro-mirrors tilt between two different positions that either direct light through the optical system (ON) or out of it (OFF). This creates a light or dark pixel on the projection surface. Each mirror can switch on and off up to several thousand times per second.

We decided on this SLM because it appeared to be capable of accomplishing our goals. The chip had XGA resolution, which means it had 1024x768 pixels, and that many pixels seemed sufficient to produce a 40x40 trap array. The DMD was also immensely cheaper than the alternatives and seemed simpler to implement than the phase modulated SLM’s, as well.

3.3 Optical Filter and Beamsplitter System (HeNe and YVO₄)

Exciting fluorescent molecules bound to biological cells requires the use of optical filters that limit the spectral characteristics of the tweezer system in a way that allows the user to see the fluorescent dyes on the cells and direct the laser to the cell at the same time. The dye that we used to view the nuclei was Acridine Orange (AO) and this dye is excited at 502 nm and fluoresces at 525nm. As was stated previously, the laser we used was a YVO₄ laser which produces mostly 1064 nm light. These wavelengths dictated the filter system that we would use. The first polychroic filter needed to reflect the 1064 nm laser and 525 nm fluorescence emission light and transmit the 502 nm excitation wavelength. It was desirable to reflect the 525 nm emission light because that is the wavelength...
at which the nuclei will fluoresce and if we illuminate the sample with this wavelength, the nuclei will not have high contrast with the background. The second polychroic filter needed to reflect 1064 nm laser light and 502 nm excitation light and transmit the emission wavelength of 525 nm. In addition to these polychroic filters there is a bandpass filter near the mercury arc lamp source of the microscope that passes 502 nm and reflects the 525 nm wavelength, as well as, an emission band pass filter near the camera which passes the 525 nm emission wavelength and reflects the excitation wavelength.

3.4 Beam Expander

Another important feature of an optical tweezer array is a beam expander that spreads out the laser beam you are using to enable spatial modulation. The YVO₄ laser beam in our system had a 325 micron waist diameter and a 2.15 mrad divergence. Our SLM has an active area of 14 x 10.5 mm with a pixel pitch of 13.68 micrometers. We want our optical beam to be as close to 10.5 mm in diameter as possible to avoid throwing away any power while utilizing as many mirrors in the digital multi-mirror device as possible. Given the lasers characteristics, it would take over two and a half meters to reach this 10.5 mm radius from the beam waist. The beam expander allows us to shrink this size down to a fraction of that length by a plano-concave lens to diverge the beam to the size we want and a plano-convex lens to recollimate the laser light. By separating the two lenses by the sum of their focal lengths you can create an expansion of your beam equal to the positive lens focal length divided by the absolute value of the negative lens focal length. In our system we placed a 300 mm focal length plano-convex lens 250 mm from a ~50 mm plano-concave lens to expand our beam from 1.5 mm to 9 mm in diameter.
A second beam expander was used in our system after the DMD in order to relay the light to the microscope and to allow us to vary the convergence of the beam entering the microscope in an attempt to improve trap efficiency. I found that this extra degree of freedom allowed me to improve the focus of the laser light at the sample but did require additional alignment.

Chapter 4  Optical System Setup

This chapter presents the techniques I used to align the optical system. The first step to setting up the laser tweezer system was to have an idea about what your system will look like. Determining where all the elements will go and what distances they need to be from one another allows you to plan where you will place these elements on an optical table.

4.1 Planning the Optical Element Locations

There were a few key factors to the optical tweezers that dictated the placement of the rest of the system. The first important factor was that the digital micro-mirror device (DMD) can only flip mirrors to on and off states that are set at specific angles to the normal of the DMD window. The on state of the DMD is at a 12 degree angle from normal and thus the DMD must be set at a 24 degree pivot in the optical system. The second factor is that the microscope does not move and really must be set in a particular position to be used. Additionally, the light needs to enter the microscope at a specific angle and
location in order to align with the optical system and pass through the microscope without being substantially aberrated. The final factor is the space that you need to have to physically place components. For instance, the second beam expander lens could not be placed too close to the DMD or the sharp reflection from the DMD would vignette on the lens mount.

### 4.2 Aligning the SLM and Turning Mirrors to Microscope

After considering the optical elements and where I would place them, I began by aligning a simple optical Gaussian beam. The order in which I placed components was determined by the degrees of freedom I had with each optical element. Placing elements that have lots of degrees of freedom after elements that have fewer degrees of freedom makes setting up the system easier. The first step to setting up the system was to set up a simple Gaussian beam that can be used as reference to the center of the optical path. First the laser is placed on the optical table in the location that seemed to be optimal after planning the placement of the whole system. The mirrors in the system are placed next because they directly alter the optical path while the lenses do not. Mirrors can be placed in the system one after another in the order that the laser would be incident upon them. In our case, the DMD will be placed first. Fortunately, our laser mounts to the table at a right angle to the side of the table. This makes positioning the DMD mirror easy because the 24 degree angle made by the incident and reflected light can be measured with the holes on the table being used as references. The next step was to turn all the mirrors to the “on” state, center the laser beam on the center of the DMD and turn the DMD so that the beam made a 24 degree angle with the incident laser. The DMD should be placed far enough away from the laser head that there is room to place the beam expander.

The turning mirror that redirects the light from the DMD is placed next and I found it easiest to place this mirror in line horizontally with the entrance aperture to the microscope. There is only one place where this scenario exists because the DMD cannot be adjusted to aim the laser at this turning mirror. In our set up we now needed a periscope to elevate the beam to the height of the microscope entrance aperture. Ultimately, the periscopes two mirrors will serve as the two mirrors that you
need to properly direct the light into the microscope at the correct angle and location. The location of the beam was adjusted with the lower mirror of the periscope and the upper mirror was used to adjust the beam angle.

After the laser beam was directed off the center of the DMD to the center of the turning mirror and close to the centers of the two periscope mirrors, the periscope was used to aim the beam into the microscope in such a way to align the laser with the microscope optical axis. In order to do this, the laser must hit the first polychroic beamsplitter in the microscope at the optical axis and with the proper angle to be redirected along the axis. I found that the best way to do this was to first approximately align the laser to the center of the beamsplitter at an angle that was approximately orthogonal to the entrance aperture of the microscope. In order to get optimal alignment through the microscope I then placed an IR viewer card on the microscope stage and viewed the brightness of the spot that was made while I adjusted the tip/tilt of the periscope mirrors. When the greatest brightness was achieved I knew I was very well aligned to the microscope. Further alignment was performed by centering the spot on the sample with the live image produced by the camera.

4.3 Aligning the Beam Expanders

Once all the mirrors are aligned to the optical axis of the microscope, the system is effectively a single trap optical tweezer. The next step is to strategically place a couple of irises to keep track of the beam location. One iris is placed about a foot behind the DMD at the positive lens focal length and the second is placed immediately before the DMD. The iris next to the DMD will be used to help align the location of the beam and the iris far from the DMD will be used to align the setup angularly.

At this point the beam expander can be placed into the optical path to blow up the beam so that more of the DMD active area can be used. The way that this was achieved was by first placing the positive lens into the system. The lens is placed first because it converges light at a far focal length so its alignment can be more easily determined than the negative lens. I placed the lens as close to the DMD as possible in order to
keep a healthy distance between the laser aperture and the first lens which was placed next. Tip and tilt of the lens and position of the lens were both adjusted until the light coming out of the lens either passed or was centered on both irises. Then the negative lens was placed at approximately 250 mm in front of the first lens. The beam was traced with an IR card or IR viewing scope to determine the collimation of the beam. The distance between the two beam expander lenses was adjusted until the beam appeared to be approximately collimated. It does not have to be perfectly collimated yet because we will place another beam expander after the turning mirror to relay the light to the lenses and resize the beam to make it easier to guide into the microscope.

Once the first beam expander was approximately aligned, the alignment was fine-tuned again by adjusting lens location until the brightest spot was created at the sample and then fined tuned more through the camera. The second beam expander was now placed in the system. Since the optical axis is once again defined by the expanded Gaussian beam, we can move the irises to new locations to use as references to where the beam should be. The first iris was placed just after where the second lens of the beam expander would be focused and the second iris was placed on a tall post around the beam as close to the microscope opening as possible.

There were some notable differences between my second beam expander and my first one. One difference is that I used a Keplerian telescope beam expander instead of a Galilean telescope beam expander. The Keplerian telescope beam expander is the same as the Galilean which was used for the first beam expander except it uses two positive lenses instead of a positive and a negative. The advantage of a Galilean beam expander is that it is more compact since you add the two lens focal lengths and one is negative and the Galilean does not produce an intermediate laser focal point. These were not issues for the second beam expander because there was plenty of room and the beam intensity was reduced and aberrated enough by that point that ionization of the air at the intermediate focal plane was not possible. Another difference between this beam expander and first one in the system is that I placed the longer 200 mm focal length lens before the 100 mm focal length lens
which actually shrinks the beam by a factor of two. I did this because this allowed the beam to be sent through the microscope without clipping.

The procedure for aligning this beam expander is the same as the previous beam expander, in that the second lens is aligned first by adjusting tip/tilt and position until the angle and location of the beam are aligned with the two irises. The first lens is placed second at a distance from the second lens approximately equal to the sum of their focal lengths. Once these lenses are aligned to the two irises, the alignment is fine-tuned by adjusting the lenses to get the brightest spot out of the objective at the sample and through the camera. The collimation of the beam is roughly adjusted by scanning the beam outside of the microscope and then the final adjustment is made by observing the spot through the camera. The distance between these last two lenses that make up the second beam expander were intended to be used to optimize the trapping spots at the focal plane of the microscope objective where the nuclei are located.

In practice, a few of these adjustments often did not directly result in better optical performance. One example of this is that when lenses were translated along the z-axis to improve collimation, often the stages they were on were not perfectly aligned with the z-axis of the optical system so the lens would become decentered and ruin the spot.
Chapter 5  Experimental Results

The purpose of our portion of this research was to achieve a large array of individually addressable optical traps capable of manipulating live cancer cell nuclei. To this end we did not reach this goal but this chapter presents the milestones we did reach.

5.1 Spot Patterns

The first step in producing an array of optically trapped nuclei is certainly to generate a pattern of microscope focused laser spots on the sample. This was accomplished using the program that came with the SLM. This program, called Discovery Explorer, allowed us to write a step-through program of patterns on the Digital Micro-mirror Device (DMD). These programs were generated by designing a pattern of spots and placing them in the order we wanted them to be displayed and for the time durations we desired. In doing so, we had expected to be able to drag cells through the sample medium by shifting the patterns
of on state mirrors across the DMD. Unfortunately, we never got far enough to test this because we did not make a stationary array of nuclei traps and we regrettably never tested the trap shift on the polystyrene beads.

Generating the patterns we wanted on the DMD was as simple as using Paint or L-Edit software to create white pixels corresponding to “on” state pixels and black regions corresponding to “off” state pixels. The real difficulty lay in directing this light into the microscope and realizing the same pattern on the sample through the microscope. This task of coherent imaging was extremely difficult and required optical elements to be spatially and angularly aligned very precisely. The difficulty of aligning the optics occasionally made it difficult to determine whether our optics were the correct distances from each other or if we were simply misaligned. In addition to the headaches caused by optical system alignment was the performance of the microscope objectives which did not seem to treat the “collimated” light equally. A sample plane pattern produced beautifully with one objective usually did not generate an acceptable pattern when another objective was rotated into the system. In general, the higher power objectives produced less desirable patterns than the lower objectives of lower magnification. The cause of this was not definitively determined but the likely reason is that the higher magnification resulted in more diffractive effects and augmentation of defects in the optics like dust and surface scratch scattering.

Unfortunately, we were unable to retrieve an optical layout for the microscope from Zeiss so that we would be able to model the system in ZEMAX and determine exactly how the incident coherent light would be manipulated by the microscope objective. This forced us to always operate on the assumption that the microscope focused collimated light at the working distance and that is a dangerous approximation for a coherent collimated light source at a wavelength outside the designated boundaries of the microscope.

Ultimately, the best DMD patterns in order to produce high quality spots were determined by trial and error. The spot patterns that I began with were simple patterns consisting of
circles and squares with various sizes and spacing. Examples of these patterns are the following:

The pattern that generated the best array was the square spot pattern shown in the figure below. My lab coworker, Matt Strathman, helped design another a spot pattern with hexagons so that all the spots would be equally spaced from one another in any direction. This hexagon pattern worked very well and is shown beside the square pattern below.

The trap patterns that appeared on the surface when the patterns above looked like the following through a 20X and 10X objective respectively:
We were also able to verify that the patterns were representative of the actual pattern on the DMD and not just diffraction patterns because we were able to address individual spots and turn them off and on.

5.2 Single Nuclei Trapping

The key to trapping cells lay in producing optical traps of the correct size and intensity. While we were unable to trap an array of nuclei we were repeatedly able to trap individual nuclei. In the series of pictures below, the trapped nuclei can be seen on the left center of the image while another nuclei passes right by. The best trapping of individual nuclei was
achieved with the DMD taken out of the optical system and replaced with a simple mirror. The minimum optical power required to trap a cell nucleus was 30 mW which corresponded to nearly 400 µW/µm$^2$. However, this power has not been optimized because no nanostructure substrates were used to enhance the field and reduce the required intensity. Ben Wilson successfully used a photonic crystal substrate to trap an ovarian cancer cell nucleus with 16 µW/µm$^2$.\textsuperscript{11}

![Figure 9: Single Ovarian Cancer Cell Nucleus Trap](image)

### 5.3 Trapped Array of Polystyrene Beads

Using the patterns shown and explained in chapter 5.1, we were able to achieve the following array of spots that were capable of trapping polystyrene beads.

![Figure 10: Polystyrene beads trapped by the optical tweezer array](image)
The beads were much easier to trap because they were smaller and have a significantly higher index of refraction than the cancer cell nuclei. The nuclei have an index of refraction that is about $1.36^{12}$ while polystyrene microspheres have an index of approximately 1.59. When compared with the index of the phosphate buffered saline solution at somewhere around $1.35^{13}$ and possibly even greater than the nuclei index. This is an important idea that I did not have the chance to fully explore while I was at UW. If the index of refraction of the medium is the same at the nuclei themselves, then the light momentum will not interact with the cell nuclei and the force generated on the cell will be small no matter how much intensity is incident on it.

The array of trapped polystyrene beads was individually addressable and we were able to turn off trap locations while leaving others on as shown in the following picture.

![Individually Addressable Polystyrene Bead Array](image)

**Figure 11: Individually Addressable Polystyrene Bead Array**

### 5.3 Cell Nuclei Trap Array

The picture below represents the best laser spot pattern I was able to achieve that had spot sizes approximately equal to the size of the ovarian cancer cell nuclei. The spot pattern was achieved with a 20x objective and the hexagon pattern on the SLM. This pattern unfortunately saw the cell nuclei pass right through without having any affect. I believe that this spot pattern could work if a photonic crystal substrate was used, the nuclei had a significantly different
index of refraction from the medium or if we were capable of delivering more power to each individual spot. Producing a pattern of this size would have been a great first step towards the large nanostructure enhanced laser tweezer array that we originally intended to produce but there still would have been many limitations of this system that will be addressed in the following chapter.

Figure 12: Attempted Nuclei Trap Array

Chapter 6 Summary and Future Work

During the course of this research, many valuable insights into the field of optical trapping of biological specimens were revealed. Although the final goal of a large array of optically trapped cancer cell nuclei was not achieved, the lessons learned can potentially aid the attempts of others to accomplish this feat.

6.1 Accomplishments

We were unable to reach our goal of a large 40x40 array of cell nuclei optical traps but we did reach some smaller milestones. We successfully trapped an individually addressable array of around thirty polystyrene beads which is perhaps the first step towards achieving the same scale of traps for cancer cell nuclei. It showed we could successfully generate an array of optical traps but the fact we could not achieve this array
with cell nuclei leads to the conclusion that our design was not efficient enough to provide the additional power necessary to trap the low refractive index cell nuclei.

Secondly, we were able to trap an individual cell nucleus and discovered some of the difficulties that would need to be circumnavigated in order to trap an array. We also determined an approximation of the power required to trap an individual nucleus with our system which yields an approximate power required to generate an array. Finally, we discovered some of the limitations of the type of optical trap array that we were attempting to create. While some may not consider this to be an accomplishment, the insight and knowledge gained from those failures may be more substantial than the knowledge gained if everything had worked out perfectly.

6.2 Limitations and Failures

There were two factors that contributed to the demise of our optical trapping system. These factors included insufficient intensity per trap and incorrect beam size and shape for traps which resulted in less efficient trapping. The most flagrant offender appeared to be a lack of power at the sample. Despite the high efficiency of the optical filters that were carefully chosen to direct the laser to the sample. The majority of the light intensity was lost at the spatial light modulator (SLM) and the microscope objective. The SLM dropped the power by an order of magnitude due to efficiency of the window coating, the nature of its amplitude modulation and diffraction effects. The digital micro-mirror device had the following transmission spectra (provided by TI):
The Digital Micromirror Device (DMD) window transmission in the figure above shows the double pass transmission of 1064 nm light to be about 90%. This is significant transmission loss but miniscule relative to the losses due to diffraction and amplitude modulation. The digital nature of on and off pixels on the DMD meant that in order to generate separate spots on our trap pattern we had to have many pixels in the off position surrounding regions of pixels in the on position. The amount of loss from this amplitude modulation depends on the pixel pattern that you use to trap. For our hexagon pattern the ratio of on to off pixels was approximately 4 to 9. That means more than half of your light is simply thrown away. The other big transmission killer is diffraction. The pixel pitch is roughly 10 micrometers and our wavelength is about 1 micrometer which means light cannot be simply modeled as a ray but experiences diffraction effects. The rows of digitally tilted pixels produced an effect akin to a well manufactured blazed diffraction grating. The diffracted orders were strong enough to be on the same order of magnitude as the specular mode. The three strongest diffracted modes contained 33, 26 and 11 percent of the power that was contained in the specular mode. Even with the SLM tilted at the correct angle to achieve a blazed
condition, the specular mode Diffraction dropped our efficiency by another large percentage resulting in a total light transmission of about 10 percent through the SLM.

The final substantial power loss was actually at the microscope objective. There were multiple objectives that we used but the most frequently used objective was the Zeiss LD EC Epiplan–Neofluar 50x/0.55 HD DIC objective. This objective was designed for optical wavelengths of light and not 1064 nm laser radiation as shown in the transmission spectrum provided by Zeiss. At 1064 nm, the transmission of the microscope objective is about 42 percent. In the end, the overall transmission of our system was around 2.5% with about 30% transmission through the microscope and less than 10% transmission through the rest of the optical system including the SLM.

Another failure of this system is the limited field size when viewing cells through the microscope. Certain microscope magnifications generated larger trapping forces than others. This was a result of the trap spot size generated by the microscope shrinking with increasing magnification and as it was shown before the optimal trap is of roughly the same diameter as the particle that is being trapped. The magnification that I was capable of generating the optimal trap for the nuclei with was the 50x objective. The picture below shows what the cells look like under the full field of the microscope using this 50X objective.
Just by looking at this picture it is easy to tell that a 40x40 array would not fit in this field of view (FOV) and even if the nuclei array could fit in this FOV, the outer regions of the FOV are hard to utilize because of aberrations in the optical system. Yet, this is the only magnification that I successfully used to trap cells. The 20x objective would allow a substantially larger FOV but I was unable to trap a cell using this objective even without the SLM in place. As was shown in the results chapter, I did manage to generate an array of spots that were about the optimal size for trapping but I saw no influence on the nuclei from the pattern.
6.3 Proposals For Future Cell Trapping

In the process of constructing this optical tweezer system, many lessons have been learned about the advantages and limitations of our current system and from these lessons a few alternative approaches have emerged as possible solutions to our current holdups. I will now discuss the alternatives that would likely allow a large nanostructure enhanced laser tweezer system to effectively function for the purpose of trapping ovarian cancer cell nuclei.

As was stated previously, the limitations of our technique is the ability to get an appropriate amount of optical power to the sample for each spot to trap a nuclei and also the inability of the microscope to generate spots of the optimal size over the entire field of view of the microscope when the objective that traps the nuclei the most effectively. An approach that remedies both of these problems is directly related to the choice of Spatial Light Modulator (SLM) and its placement within the system. For our system we chose an amplitude modulated digital micro-mirror device (DMD) to modulate the light into individually addressable spots. We have shown that the DMD is incredibly inefficient and difficult to work with because of uncontrollable diffraction and loss of light.

The alternative to an amplitude modulated SLM is a phase modulated SLM. The most common type of phase modulated SLM is the liquid crystal phase modulator in which liquid crystals are used to retard
the phase of the coherent plane wave before it is imaged to a certain pattern. This technique requires complex numerical calculations in order to generate the spot pattern that you desire at the sample but also gives you incredible flexibility to create the spots you want precisely. The phase-modulated SLM’s also do not experience the problems that were discussed related to the amplitude modulated SLM. There is no loss of light due to “off” state pixels because the spots are generated from an in-phase coherent light or constructive interference while the dark areas in the pattern are where light is destructively interfering. Additionally, power loss due to diffraction effects can be reduced using the SLM with high diffraction efficiency. You will still experience reflections and scattering from the phase-modulated SLM but the efficiency will still be vastly superior to the amplitude modulated version.

Using a phase modulated SLM would probably necessitate bringing light to the cell sample from the bottom of the sample instead of through the microscope and hitting the cell nuclei from above. This is actually beneficial to the efficiency of the system because using additional optics to focus the phase modulated light at the sample from the underside avoids sending 1064nm light through the microscope objective which cuts down the light transmission drastically, as well. By placing a lens underneath the sample and placing an IR filter above the sample you would avoid sending intense infrared light into the microscope where it could be potentially damaging to someone’s eyes or even the objective itself and also subject the cell nuclei to enough power to be trapped. This set up would also be practical for enhancing the optical traps with transmissive photonic crystals to reduce power needed at the trapping sites.

Yet another benefit of sending laser light in from beneath the sample and decoupling the trapping optics from the sample viewing optics is that you can increase the size of the array you would like to generate so that it is not limited by the microscope field of view and aberrations in the larger field regions. However, in order to utilize and view the whole trapping region would require that the optics generating the trap and the translation of the sample be coupled. This would be difficult with the current system because the microscope objective does not move but rather the sample is moved under the objective. Regardless of whether the regions beyond the objective field of view are utilized you would increase the region of trapping within the microscope field of view by bringing the light in from below.

The final advantage of bringing light to the sample from below the microscope is that you would then know exactly what optical elements you are working with. There would be no mysterious microscope objective to send light into and pray that it produced the correct
results. Knowing the exact optical system within certain tolerances would have allowed us to model the system much more accurately and determine exactly where our optical elements needed to be.

Utilizing a phase modulated SLM would likely be enough to produce a highly efficient and effective large individually addressable optical tweezer array and there is one other suggestion I will make on that end to improve the performance of this system. That is to enclose the optical trapping system to remove dirt and debris that can generate scattering which not only can decrease optical efficiency but destabilize the optical traps themselves. A lab in the University of Washington Department of Physiology and Biophysics using an individual optical tweezer setup had enclosed their system and noticed a dramatic improvement in trap stability after the alteration was made. Enclosing the optical trap may prove to be especially crucial when a larger trap array is generated and the laser intensity in the outer field regions is less intense.

![Figure 17: Phase Modulated SLM's Sold by Holoeye](image17)

One final idea is for our collaborators to try to decrease the refractive index of the PBS solution or use a different medium of lower index if possible so that light is refracted more through the nuclei and the trapping force is greater for the same amount of incident intensity.
Bibliography