PHYS 39a Lab 3: Microscope Optics

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Abstract

In this lab task, we sought to use critical illumination and Köhler illumination techniques to view the image of a 1000 lines-per-inch metal foil grid and compare the resulting image qualities. We also used an optical lens setup as a means of constructing the spatial Fourier transform of the sample on the focal plane of a convex lens. Various components were allowed to be inverse-transformed, therefore allowing one to explore the spatial structure of the sample in a two-dimensional frequency space.

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Theoretical Background

Geometric Optics In the thin-lens approximation, an ideal lens will take parallel light rays and refract them towards a single point, called the **focal point**. We can characterize convex lenses by the distance between the lens and its focal point, which we call the **focal length** f, as pictured in Figure **??** (left). The locus of points one focal length away from a lens is called the **focal plane**. Due to the symmetry of the lens, light that passes straight through the middle of the lens must be left unrefracted, so the intersection of a light ray passing form an object at a distance greater than one focal length away from the lens and a parallel ray refracted by the lens (and therefore passing through the focal point) exactly define the location of the object's **real image**, as pictured in Figure **??** (right). If we call the horizontal distance between the object and the lens o and the horizontal distance between the lens and real image i, a simple comparison of similar triangles gives us what is called the **thin lens equation**, which relates o and i to the focal length for such idealized lenses.

$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f} \tag{1}$$

By inspection, we can also see that the relative size of the real image to the object is given by its **magnification** M, which is defined as follows.

$$M = \frac{i}{o} \tag{2}$$

Spherical Aberration The most cost-effective manner in which to construct a lens is by using spherical sections of material, as suggested by the 2D schematic in figure **??**. Unfortunately, it turns out that such lenses do not perfectly focus light according to the idealization required to generate the thin lens equation. It turns out that instead of refracting parallel rays to a single focal point, spherical lenses refract parallel rays to numerous focal points. The further away an incident light ray is from the center of the lens, the further away from the idealized focal point the refracted ray is oriented. This particular deviation from idealization is called **spherical aberration** (aberrations in general refer to refractory deviations from lens idealization) and is represented by the diagram in Figure **??**.

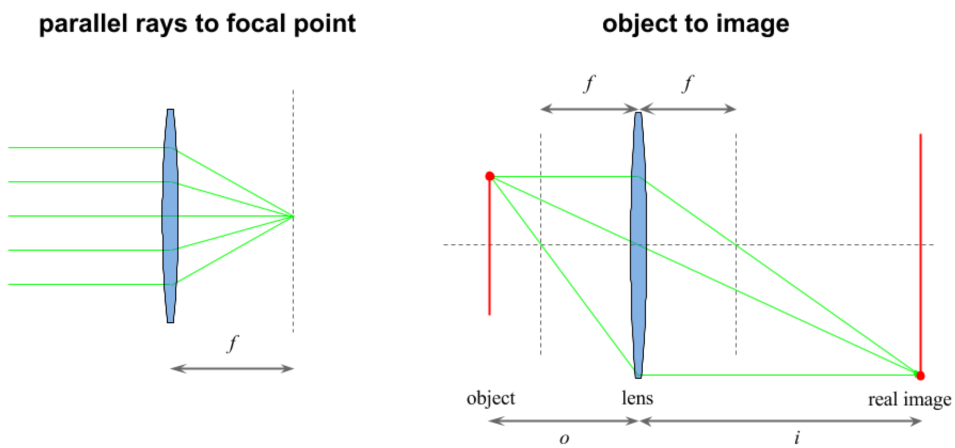

Figure 1: Diagram displaying the ideal thin lens' manner of refracting parallel light rays towards a single point (left) and point sources to single points (right)

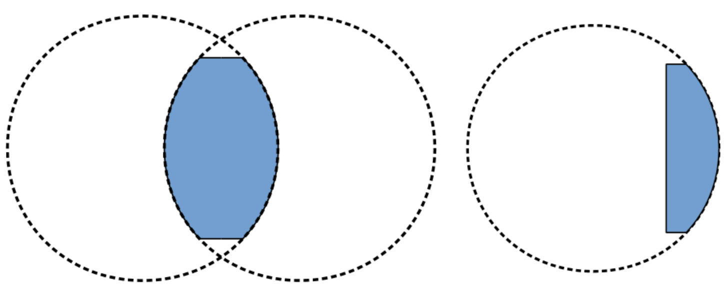

Figure 2: Shematic diagram of various ways a convex lens can be formed out of spherical sections.

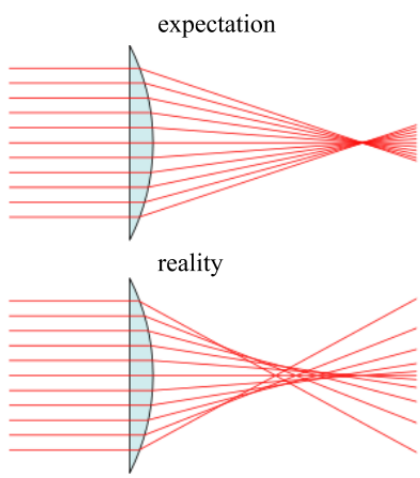

Figure 3: Diagram depicting the refractory deviations present in cases of spherical aberration.

One of the most common ways to troubleshoot the effects of spherical aberration are to use a variable-diameter aperture to block out marginal rays so that the remaining light rays form more approximately a single point in the focal plane. This technique is schematically depicted in Figure **??**.

In this experiment, we had three primary goals:

1. Use critical illumination to view the image of a sample.

2. Use Köhler illumination to view the image of a sample and compare to critical illumination.

3. Use principles of Fourier optics to physically generate a spatial Fourier transform of a sample.

Experimental Setup

During this lab task, we constructed three main types of setups to create various images of a sample: critical illumination, Köhler illumination, and Fourier optics. The sample we used was a 1000 lines-per-inch metal foil grid.

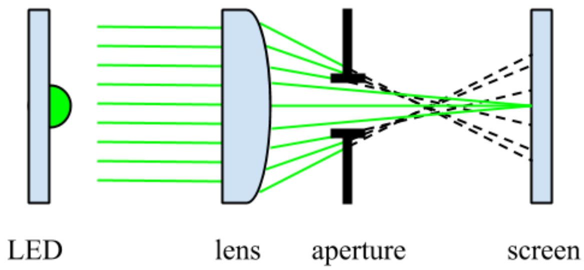

Figure 4: Shematic representation of how to reduce the effects of spherical aberration using a variable-diameter aperture. The dotted black lines represent light rays that are

Critical Illumination Critical illumination is a technique wherein light from a light source is directly focused into a microscope objective, with the sample placed extremely close to the microscope objective. Therefore, only the light that passes through the holes in the sample will be recorded by the camera. The following components were aligned and laid out in a line in the following order in order to achieve critical illumination.

1. LED light source
2. O.D.1 light filter [1]
3. Variable-diameter aperture, to reduce effects of spherical aberration
4. DCX25 (25 mm focal length convex lens), positioned as to focus LED light into microscope objective
5. Metal foil grid sample
6. Microscope objective
7. Camera, positioned to capture light from objective

[1]Reduces source light amplitude to prevent camera sensor saturation.

Köhler Illumination Köhler illumination is a technique wherein light from a source is greatly unfocused while still allowing the image of the sample to remain in focus for the camera. The general goals of employing Köhler illumation was to eliminate the image of the light source from the sample plane and to limit stray light that could cause lens flare or interact with structural components of the microscope. The latter effect is achieved through the use of two apertures, called the field diaphragm and aperture diaphragm which limit light illuminating non-imaged portions of the sample and limit light illuminating the sample from extreme angles. The following components were aligned and laid out in a line in the following order in order to achieve Köhler illumination.

1. LED light source
2. DCX25 (25 mm focal length convex lens), positioned as to focus LED light into second aperture, called the "collector lens"
3. Variable-diameter aperture, called the "field diaphragm," positioned in the plane that will be focused onto the sample through the second condensor lens
4. Variable-diameter aperture, called the "aperture diaphragm," positioned in the focal plane of the LED through the collector lens
5. DCX25 (25 mm focal length convex lens), positioned as to focus the field diaphragm plane onto the sample.
6. Metal foil grid sample
7. Microscope objective
8. Camera, positioned to capture light from objective

Figure **??** demonstrates a Köhler illumination setup without the field diaphragm or the aperture diaphragm put in place. Here, red rays indicate stray light from the LED and green rays indicate LED rays angled appropriately on the sample. The pink vertical line represents the sample and the grey bars indicate the section of the sample that is viewed by the camera. First, putting in the aperture diaphragm, as shown in Figure **??**, blocks out the stray light rays (red). Second, putting in the field diaphragm, as shown in Figure **??**, blocks out the appropriately angled light that does not illuminate the target portion of the sample.

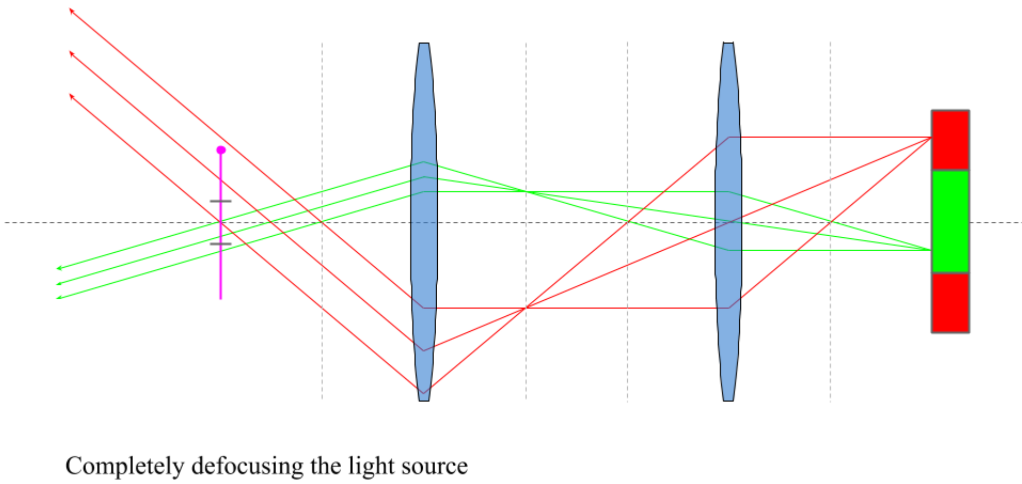

Figure 5: Schematic representation of the Köhler illumination setup.

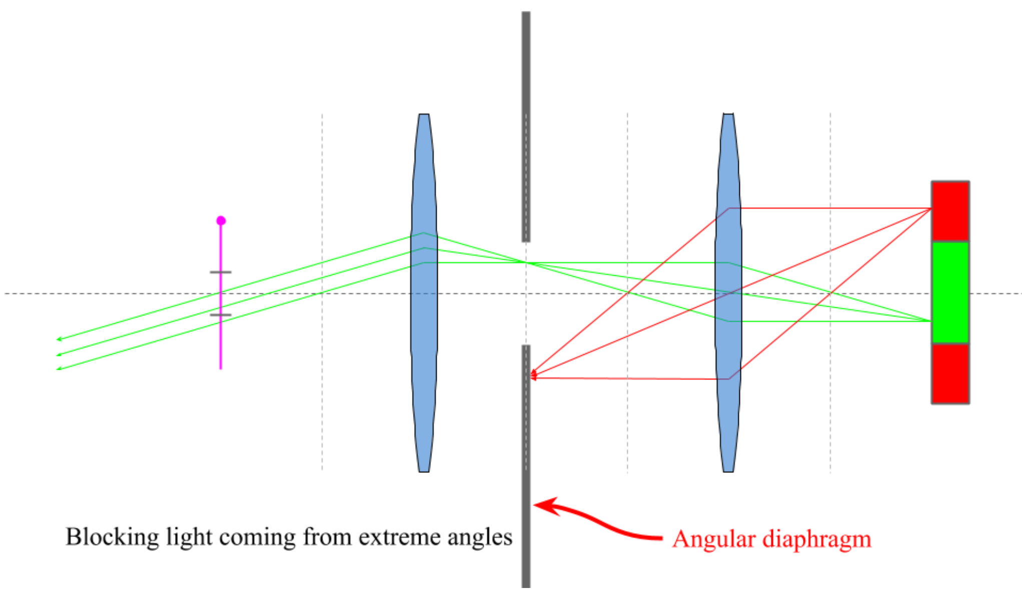

Figure 6: Insertion of an aperture diaphragm.

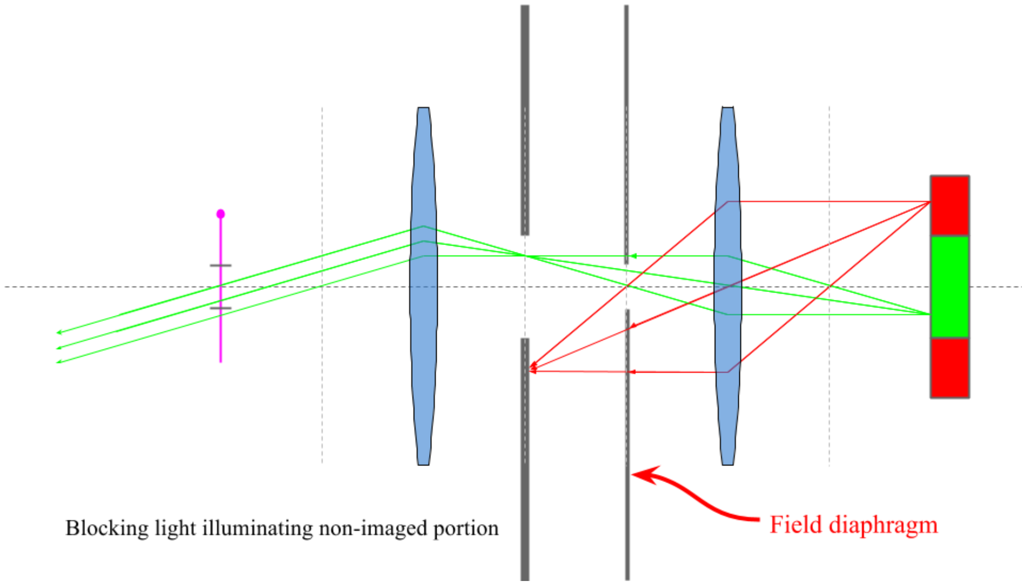

Figure 7: Insertion of a field diapraghm.

Fourier Optics It is of significant coincidence that the diffraction nature of light corresponds exactly to a spatial Fourier transform with an angular parameter. A convex lens is then able to focus the resulting light to generate a 2D representation of the Fourier transform in the lens' focal plane.

Consider in two dimensions an arbitrary sample. We can describe the sample entirely in terms of a complex-valued "transparency" function $T(y)$ which is defined such that if the electric field incident on the sample is $E(y,t)$, the electric field that exits the sample on the other side is $T(y)E(y,t)$. In the simple case of an opaque metal foil grid, the $T(y)$ function will equal 1 at all of the "holes" and 0 elsewhere. Consider a plane wave $E(y,t) = E_0 e^{i\omega t}$ incident on the sample. The electric field that exits the sample on the other side is then

$$E'(y,t) = E_0 T(y) e^{i\omega t}. \tag{3}$$

Consider an point P whose straightline connection to the sample creates an angle θ with the incident plane wave, as shown in Figure **??**. Each point on the sample plane can be treated as a point source by the Huygens-Fresnel principle. Therefore, we can calculate the electric field at P by integrating across the entire sample, treating each point as a point source, and accounting

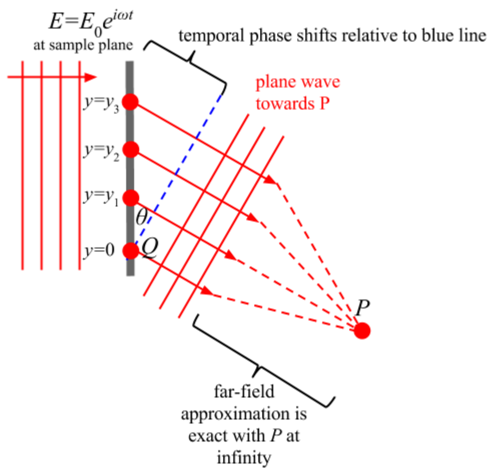

Figure 8: Light diffracted through an arbitrary sample oriented towards an arbitrary point P.

for the appropriate phase shifts as given by the far-field approximation (which no longer ends up as an approximation when P is set at infinity). The far field approximation takes a geometric approach to calculating the phase shifts, stating that relative to a point Q, which we will put on the sample at $y = 0$, the phase shift of light emitted from a Huygens-Fresnel point at a position y will have a phase shift of $\frac{y}{c}\sin\theta$. This is exactly the amount of time taken for light to traverse the red paths connecting the blue dotted line to the sample in Figure **??**. Therefore, the electric field at point P becomes

$$\begin{aligned}
E(P) &= \int_{\forall y} E'\left(y, t + \frac{y}{c}\sin\theta\right) dy && (4)\\
&= \int_{\forall y} E_0 T(y) e^{i\omega(t+\frac{y}{c}\sin\theta)} dy && (5)\\
&= \int_{\forall y} E_0 T(y) e^{i\omega t + ky\sin\theta} dy && (6)\\
&= E_0 e^{i\omega t}\int_{\forall y} T(y) e^{i(k\sin\theta)y} dy && (7)\\
&= E_0 e^{i\omega t}\mathcal{F}_y[T(y)](k\sin\theta), && (8)
\end{aligned}$$

which is proportional to the Fourier transform of the sample's complex trans-

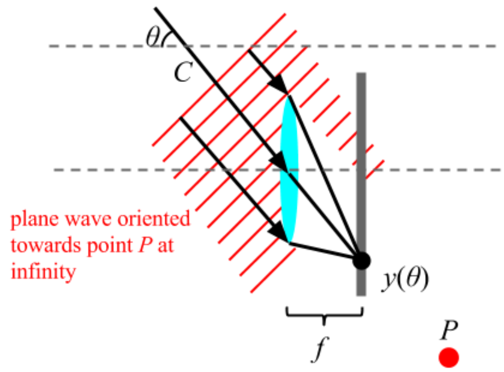

Figure 9: Every plane wave is focused by a convex lens onto the lens' focal plane located at one focal length f away from the lens at a unique point $y(\theta)$ determined by the plane wave's angle of incidence θ.

parency $T(y)$ with frequency parameter $k\sin\theta$, written as $\mathcal{F}_y[T(y)](k\sin\theta)$. Therefore, the intensity of the resulting light (i.e., the brightness of the resulting light) will be proportional to the square of the modulus of this Fourier transform, $|\mathcal{F}_y[T(y)](k\sin\theta)|^2$.

Finally, using the principles of geometric optics, we can see that convex lenses will map a plane wave at a particular angle to exactly a unique point on its focal plane. Therefore, each component of the Fourier transform of the sample is assigned a point in the focal plane, meaning that the intensities of the light present in the focal plane give an exact representation of the square of the modulus Fourier transform of the sample. This focusing can be visualized with the aid of Figure **??**.

Experimental Findings

For each of these experiments, we looked at the image of a 1000 lines-per-inch metal foil grid.

Critical Illumination of Sample Overall, the image produced by critical illumination (as described in the Experimental Setup section) ended up less

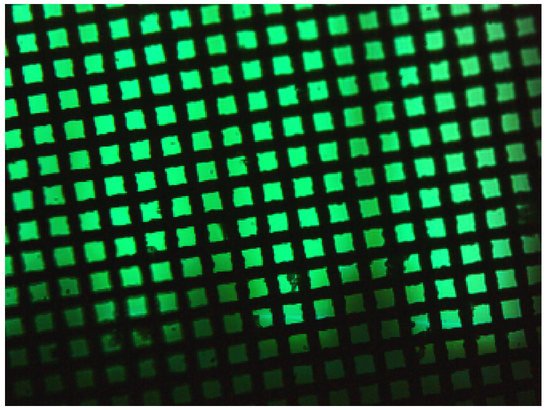

Figure 10: Image of sample under critical illumination.

than ideal. A general shot is displayed in Figure **??**, where it is clear that the illumination is not uniform, due to the non-uniformity of the LED light source. This is a side-effect of the plane of the LED light source being almost in focus to the camera, effectively allowing the LED light source to itself form a defocused image to the camera.

We used the a variable-diameter aperture placed between the LED and the convex lens to reduce the effects of spherical aberration. However, when this aperture was closed, we found that the sharpness of the image was decreased, the contrast of the image was decreased, and diffraction fringe effects became more pronounced. It is because of these effects that utilizing Köhler illumination becomes advantageous. Images of the sample under critical illumination for various aperture sizes is displayed in Figure **??** where these effects can be seen.

Köhler Illumination of Sample Köhler illumination proved to be much more practical and useful in terms of accurately viewing the sample. The configuration of optical components was set up as described in the Experimental Setup in order to generate a clear image of the sample that reduced stray light and forced the source light to be completely out of focus.

Fourier Decomposition of Sample Using a laser light source, we were able to diffract light through the sample as described in the Experimental

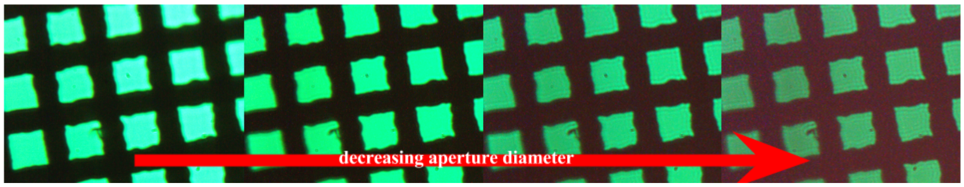

Figure 11: Image of sample under critical illumination for various aperture sizes intended to counter the effects of spherical aberration. The red arrow indicates a decresaing aperture size.

Setup section for Fourier Optics. The focal plane of the convex lens we placed directly after the sample generated the image visible in figure **??** on its focal plane, which was viewed by inserting a piece of paper along this plane. This pattern represents the square of the modulus of the spatial Fourier transform of our sample.

We compared this observed Fourier transform to one computed in Mathematica as well as closed an aperture in the Fourier plane to only allow certain components through and placed another convex lens one focal length away to generate an inverse transform that was then viewed through the camera. We used Mathematica to also generate analogous inverse transform images. All of these results are summarized in Figure **??**. Note that the middle "dot" corresponds purely to background noise and contains no information about the repetitive structure of the original sample.

We also decided to let through only specific Fourier components to explore the resulting inverse transforms. Points in the Fourier plane that are opposing to each-other with respect to the origin correspond to complex conjugate pairs, so their superposition generates a real-valued waveform that is then visible on the inverse transform plane as sine and cosine waves. These components, when displayed without their conjugate pairs, provide a featureless inverse transform, similar to what is seen in the inverse transform plane when only the center "dot" is allowed through. This is because functions of the form e^{iky} and e^{-iky} have constant modulus with respect to the parameter y, but their superposition $e^{iky} + e^{-iky} = 2\cos(ky)$ has a non-constant modulus.

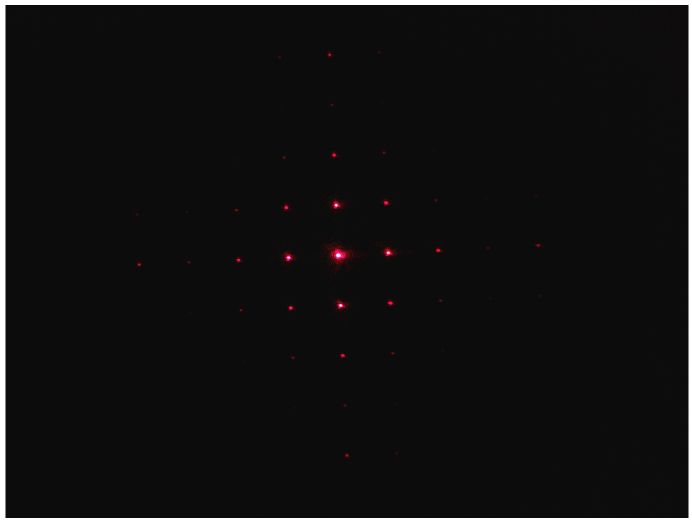

Figure 12: Physially generating a Fourier transform of our metal foil grid sample.

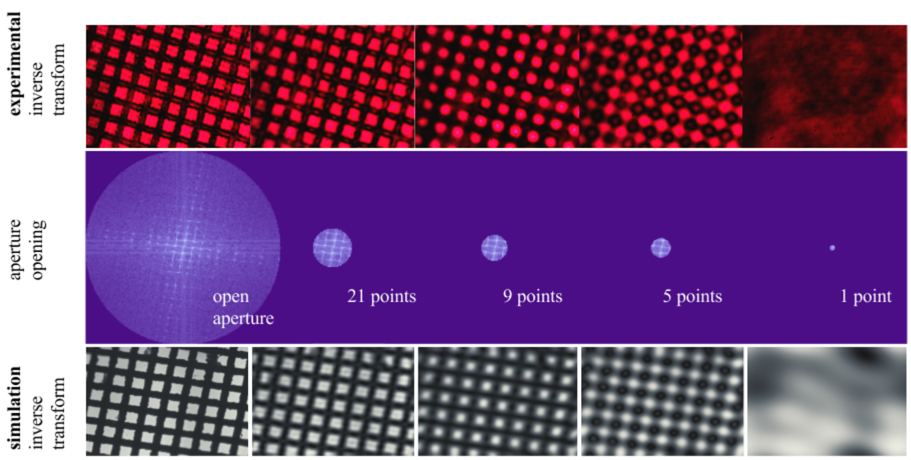

Figure 13: Resulting inverse transforms and Mathematica simluations for various aperture sizes in the Fourier plane.

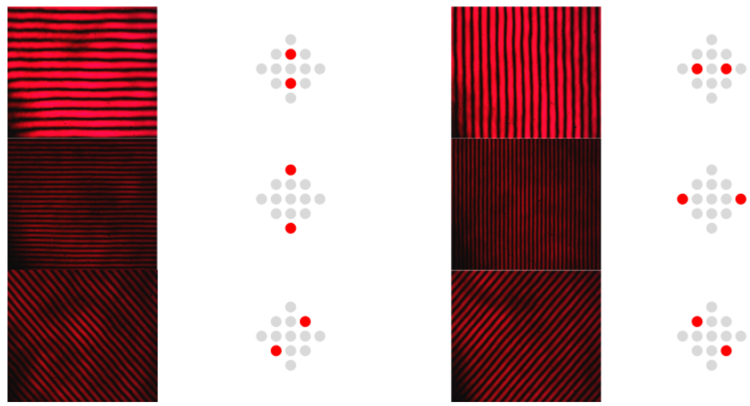

Figure 14: Allowing only particular components to shine through revealed interesting patterns. Note: opposing dots correspond to conjugate pairs.

Conclusion

Real-World Relevance Microscopes are devices that are used widely in the sciences and use a combination of lenses and various lighting methods to generate images of samples in the same way that we have here. Constructing images using optical components gives me a huge appreciation for the amount of work and research that has gone into creating reliable and useful lighting techniques, as well as the amount of effort necessary to fine-tune the positioning and constructions of microscope setups.

Diffraction and interference are very fundamental principles in studying the wave-nature of light and quantum mechanics. The results of Fourier Optics, such as the aspects that we have explored here in this lab task, are very profound and serve as an incredible example of the manner in which light can interact with itself and produce very detailed patterns corresponding to highly intricate mathematical correspondences.

Advice to Future Lab Students Various pieces of advice for future students pursuing this experiment:

- Be very careful in the initial alignment of the optical components. Misalignment will not allow intended light to be directed into the camera.
- Do not get too intricate with calculations with where along the track to

put the optical components exactly. Frequently it turns out that manual adjustments are necessary in the end anyway, so a rough estimate is frequently all that is necessary to get one on the right track towards generating a clear image.

- This is a highly recommended lab because the part dealing with Fourier Optics is way cool!

Suggestions for Improvement Various suggestions for improvement of this lab experiment:

- The lab felt more like a "task" as opposed to an "experiment" in that the driving motivation was to create something as opposed to solve a problem, so while it was physics-based, it didn't feel very scientific. For instance, there were no hypotheses to be generated.