Using Fourier optics for spectrometry and dark-field microscopy.

Third Year Lab Report

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(Dated: December 13, 2023)

In this experiment, light from an LED is shone through a series of lenses and irises, forming a Köller illumination system to light an object. The diffraction around the object in the object plane forms a Fourier plane (FP) in which the diffracted light is described by a Fourier transform. As such, the light moving through the FP is limited in ways which achieved simple spectroscopy and dark-field microscopy to a diffraction limited resolution in blue wavelength of (740 ± 34)nm and in red, (1019 ± 37)nm.

1. INTRODUCTION

The experiment consists of the formation of a rudimentary microscope which uses an object (opaque shapes on transparent glass) to generate an image onto a camera lens. Between the objective lens and tube lens lies the Fourier plane, an area in which the light intensity is described by a Fourier transform of the object. Due to the nature of the Fourier transform, the function describing intensity is transformed into one which describes the spatial frequencies (a characteristic that is periodic in position) present in the original object. Masks of varying shapes were applied to the FP to achieve several results by manipulating repeating spatial properties in the original function, such as lattices and clarity, which led naturally to the filtering and image editing necessary to form a simple spectrometer and to achieve dark-field microscopy.

The simple spectrometer allowed the calculation of the wavelengths of a range of colour filters, which could then be used in dark-field microscopy experimentation to improve the diffraction-limited resolution of the microscope. Dark-field microscopy is a form of microscopy in which the background is darkened and the details of the object are illuminated. This is done through the introduction of a mask in the FP which eliminates all but the diffracted light. As a result, the field around the object is darkened. This is a method of microscopy frequently used in biology as it can help with the viewing of live or unstained subjects.

2. THEORY

Fourier optics is a phenomena emergent from diffraction and interference. In its simplest, the Fourier transform of a function describing a narrow slit is seen in the amplitude of the light diffracted onto a distant screen through the slit [1]. Diffraction is described by Kirchhoff's diffraction formula [2], where in the far-field (Fraunhofer), the complex amplitude of the diffracted wave U takes the form of

$$U(x,y,z) \propto \int\int_{Aperture} A(x',y') e^{-i\frac{2\pi}{\lambda z}(x'x+y'y)} \mathrm{d}x' \mathrm{d}y' \tag{1}$$

where A is the complex amplitude of the illuminating, monochromatic wave of wavelength λ arriving at the aperture in the $x'y'$ plane. This, mathematically, is a Fourier transform in two dimensions at spatial frequencies $f_x = x/(\lambda z)$ and $f_y = y/(\lambda z)$ [2].

The introduction of lenses allows the far-field Fourier plane to be brought to the focal plane of the lens, giving lenses the property of being able to perform a Fourier transform. Thus, a system of two lenses with equal focal lengths f (a 4f processor) can be created to project the image plane onto a camera while the FP lies directly between at distance $2f$ from from the object (f from either lens) as shown in figure 1. This allows the manipulation of the light directly in the FP, giving rise to a multitude of filtering and image editing effects when limiting the spatial frequencies which are allowed to arrive at the camera.

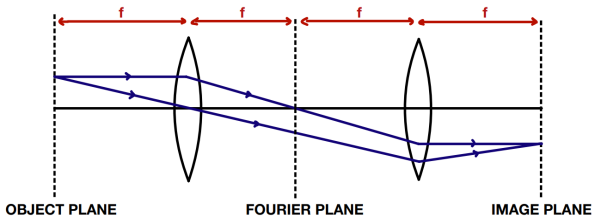

FIG. 1. A diagram which shows two ray paths between the object plane and the image plane in the 4f processor setup, with the Fourier plane lying between.

The diffraction limited resolution of a microscope (the minimum distance between two points which can be resolved) is described by Abbe's diffraction limit,

$$\Delta x_{min} = \frac{0.61\lambda}{\mathrm{NA}} \tag{2}$$

where λ is the wavelength of the light and NA is the numerical aperture, a term which describes the range of angles over which the system can accept incoming light rays and follows the relation $\mathrm{NA} = n \sin\alpha$ where n is the refractive index of the medium surrounding the lens and α is the angle of the marginal ray from the normal of the lens [3]. A microscope with a smaller value of Δx_{min} is able to resolve objects which are closer together, and thus has a better resolution.

3. METHODOLOGY

The majority of the experimental results were obtained from a system forming a microscope. The microscope is broken down into several smaller components, each of which are made from several lenses and other optical components. The Illuminating optics, forming a Köhler illumination setup, is made from a collector lens, field diaphragm, field lens, aperture diaphragm and condenser lens. The collector lens lies close to the light source, and projects it onto the aperture diaphragm, which lies in the focal plane of the

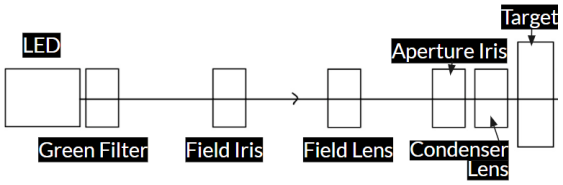

FIG. 2. The Köhler illumination system used in the experiment.

condenser lens. The condenser lens then acts to then project this light through the object. Adjusting the aperture iris (diaphragm) changes contrast, analogous to changing the aperture on a camera. This alters the effective numerical aperture (NA) of the microscope and adjusts the angles of marginal rays from the light source such that they land on the objective lens while keeping the illuminated area the same. Next to the collector lens is the field iris, the image of which lies in the object plane, making it possible to change the area of illumination without changing the angle of the marginal rays. Finally, the field lens simply acts to allow better focusing and increased intensity in the object plane [4]. This setup in the experiment is shown in figure 2 and exists to suitably illuminate the object.

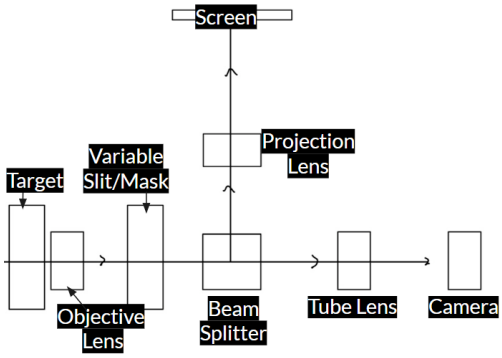

FIG. 3. The imaging optics setup used in the experiment.

The object lies in the object plane which is followed by the imaging optics system as shown in figure 3. The illumination on the object causes light to diffract around it and be passed through the objective lens. The objective lens and tube lens form something similar to a '4f processor' as discussed in section 2. The main difference is that the focal lengths of the lenses are no longer identical. Our first lens, the objective lens, had a focal length of 30mm and the second, the tube lens, had a focal length of 150mm. Using the equation

$$\text{Magnification} = \frac{f_{tube}}{f_{objective}} \tag{3}$$

where f_{tube} and $f_{objective}$ are the focal lengths of the respective lenses it can be shown that in our setup the magnification of the microscope was 5 [4].
Additionally, joined to the end of the LED was a green filter, allowing a greatly reduced range of wavelengths to the camera and eliminating the effects caused by chromatic aberration, which is where different wavelengths are refracted by different amounts in lens systems. This refraction in a single slit system is governed by the equation

$$a \sin\theta = m\lambda, \tag{4}$$

where a is the size of the slit, θ is the angle of the maxima from the normal of the plane containing the slit, m is the maxima order and λ is the wavelength of the light [5]. In the far-field limit this is used to approximate the fringe spacing y as

$$y = \frac{m\lambda D}{a}, \tag{5}$$

where D is the distance to the screen from the slit. Importantly, this shows that $y \propto \lambda$.

4. RESULTS

The cameras from Thorlabs have uncertainty on any distance measurement of 3.45µm due to the physical size of the pixels [6]. Additionally, the Thorcam software required to use the cameras introduces an unavoidable source of human error, as there is a limited number of program tools for measuring physical distances. The measurements must be done partially 'by eye', with no guarantee that lines drawn in the software are parallel to the lattice variation being measured. These two sources of random error are reduced easily when measuring equally spaced, repeating fringes, as the distance between multiple maximas can be measured at once, reducing error by a factor of the number of fringes measured.
When working with filters, the largest source of error comes from the Gaussian distribution of light passing through the filter provided with Thorlabs kit used in this experiment. The green filter had an associated FWHM (full width at half max) of 40nm [4], which through the equation

$$\sigma = \frac{1}{2\sqrt{2\ln 2}}\Delta_{FWHM}. \tag{6}$$

is used to calculate the error on wavelength. This means that for use in calculating uncertainty of results, the wavelength λ of the filter is (550 ± 17)nm.

4.1. Qualitative Results

A large portion of this experiment returned results that cannot be shown through numbers. Much experimental image editing was done through various masks as to show and discuss the physical effects of several processes. Early experiments showed the Fourier transforming properties of the lenses. Through this line of experimentation it was found that masking every other maxima in the FP would half the distance between the resulting structure in the image plane.
Additionally, Babinet's principle was verified. That is to say that apart from in the zeroth order maxima, the diffraction patterns of complimentary structures are indistinguishable [5]. This was shown by masking the zeroth order in the FP for an object containing a structure and its compliment (opaque where the original was transparent and vice versa).

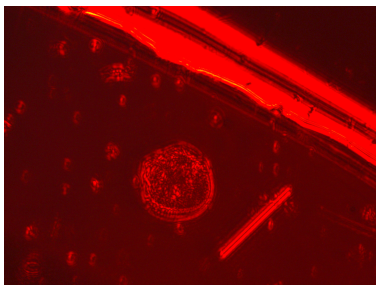

FIG. 4. The camera view of a diatom slide under red light in the microscope. The objective lens is purposely slightly out of focus to show the effects of changing the filter colour in figure 5.

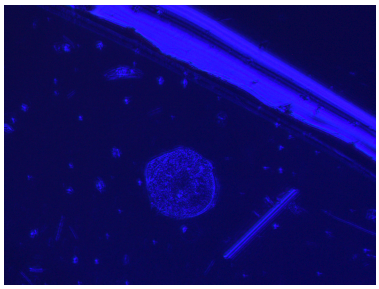

FIG. 5. The same diatom slide from figure 4 under blue light in the microscope.

Using equation 5 in the form $y = k\lambda$ and the known-wavelength green filter, it was found that the constant of proportionality, k, was $(213.8 \pm 6.7) \times 10^6 \text{pxnm}^{-1}$ (pixels per nanometer) through changing the location of the camera to the FP and measuring the fringe spacing which resulted from a narrow slit placed in the object plane.

4.2. Simple Spectrometry

Filter Colour	Wavelength (nm)
Red 1	700.0 ± 26.0
Red 2	620.2 ± 19.9
Orange	594.0 ± 18.8
Green	539.3 ± 17.1
Blue	450.4 ± 19.2

TABLE I. Table showing the wavelengths found through simple spectrometer of various colour filters.

Then, by finding the fringe spacing, y, for each of the other colour filters, the wavelength at which they filter was found, as shown in table I. All the results found give wavelengths with reasonably errors in regions of the visible light spectrum that are expected for each of the colours [3]. The 'Red 1' filter has a higher degree of uncertainty due to it allowing less light overall to pass through, making it much more difficult to make out the individual fringes for the same settings in the camera and so adjustments were made reducing the accuracy of measurement

4.3. Dark field Microscopy

Filter Colour	Resolving Power (nm)
Red 2	1019 ± 37
Blue	740 ± 34

TABLE II. Resolving power for two of the filters from table I which were used to find if our microscope setup was diffraction limited.

Using the wavelengths found in table I for each of the filters and Abbe's diffraction limit from equation 2, it is known that bluer, higher wavelength light has a better resolution in dark-field microscopy. However, finding respective resolving powers as shown in table II demonstrated that this diffraction limited resolution was smaller than the resolution of the camera, proving that our microscope setup was not diffraction limited.

For demonstration's sake, the objective lens could be slightly unfocused to blur the camera image, so that the changing of the filter on the light visually showed an improvement of clarity in the blue compared to the red. The change between figures 4 and 5 is subtle but noticeable.

5. DISCUSSION

An interesting takeaway from Fourier optics is the property of lenses allowing them to perform a Fourier transform as mentioned in 2. Naturally, this process is performed as fast as the light propagates and is able to diffract and interfere, therefore making a lens a much faster optical processor than any single computer. Phase information is lost in the measurement of the result however, but could be retrieved in an extension to this experiment through interferometry.

6. CONCLUSIONS

The results in this experiment clearly show the ability to use Fourier optics in a spectroscopic capacity, which led to the result of improving clarity in dark-field microscopy by using higher-frequency, bluer light. This was quantitatively shown through calculations of the resolution in a red and blue scenario of (1019 ± 37)nm and (740 ± 34)nm respectively. Additionally, the Fourier-transforming properties of lenses and Babinet's principle were qualitatively verified.

[1] T. Young, Phil. Trans. R. Soc. **94**, 1–16 (1804).

[2] E. Hecht, *Optics*, fifth edition. global edition. ed. (Pearson Education, Inc., Boston, 2017) pp. 457–587.

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[4] Thorlabs, *Fourier Optics Kit Manual* (2022), EDU-FOP2/M.

[5] M. Born and E. Wolf, *Principles of optics*, seventh (expanded) anniversary edition, 60th anniversary edition. ed. (Cambridge University Press, Cambridge, 2019).

[6] Thorlabs 1.6 MP CMOS Compact Scientific Cameras feature page, `https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=13677`, accessed: 07/12/2023.