Lab Write-Up Week 5

Kohler, Conjugate Planes, and Darkfield

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Darkfield Illumination

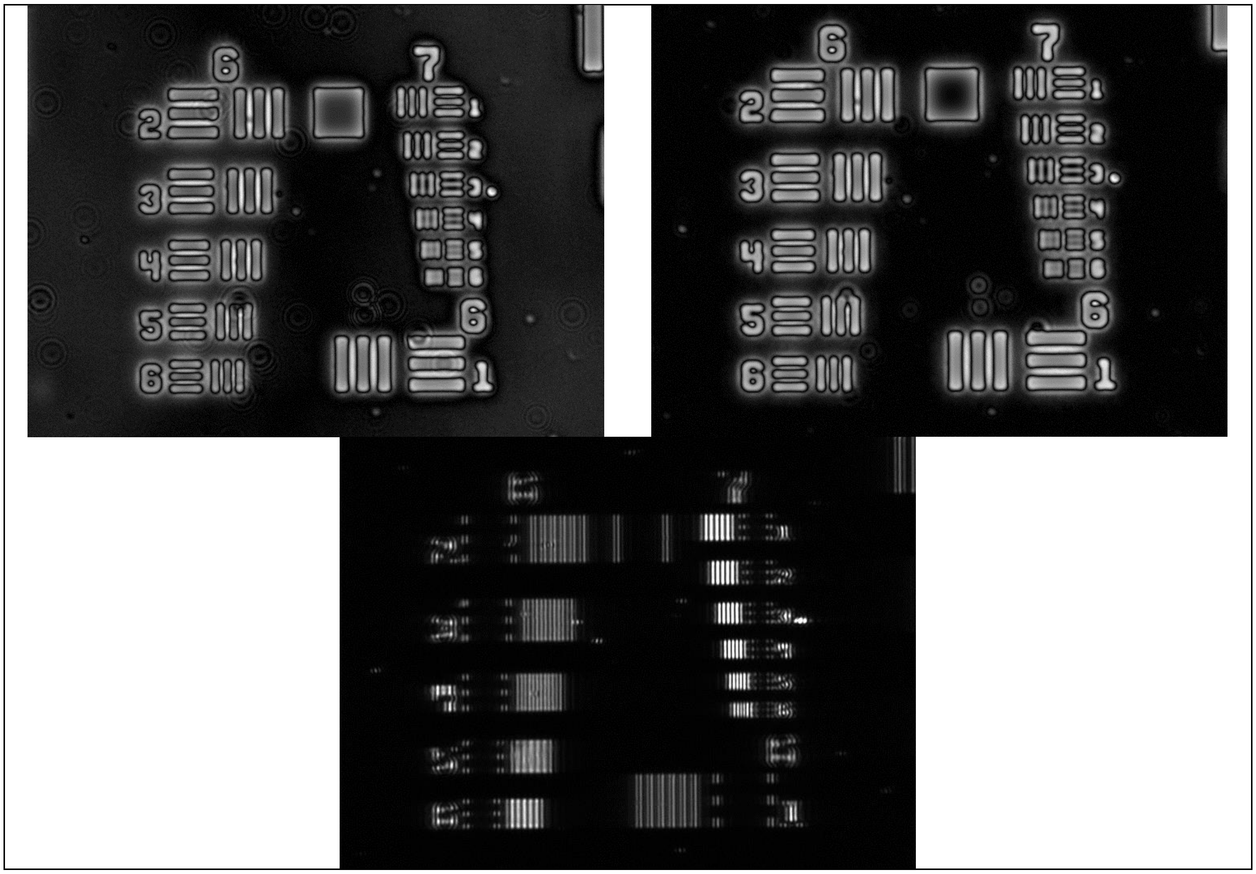

Figure 1. (Left): Darkfield image created by subtracting image with 7.5mm BFP diameter and an image with 1mm BFP dia. (Right): Darkfield image create by taking an image with 7.5mm BFP diameter and subtracting the same image with a Gaussian convolution applied to simulate a smaller BFP aperture size.

Understanding that resolution is dependent on the numerical aperture of a lens, which is varied by changing the iris diameter of that lens; an image taken with a larger aperture will have higher resolution than an image with a smaller aperture. The higher resolution image can resolve smaller features and can be said to contain higher spatial frequencies that the lower resolution image lacks. Subtracting the low-resolution image containing low spatial frequencies from the better image leaves only the high spatial frequencies in the resulting image, appearing as the small features being illuminated and the background being dark.

'blurriness' in an optical system is caused by its PSF, where a single point on the objected is mapped to a radius in the imaging plane. These point spreads, or airy disks, overlap each other and are convoluted together to form an image. A Gaussian convolution is a close approximation to an PSF, that is a Bessel function. Applied to a high-resolution image, the Gaussian convolution creates an image that is very similar to that of an image taken with a lower NA. Such that the result of subtracting the 1mm BFP image and the convolution image have similar results.

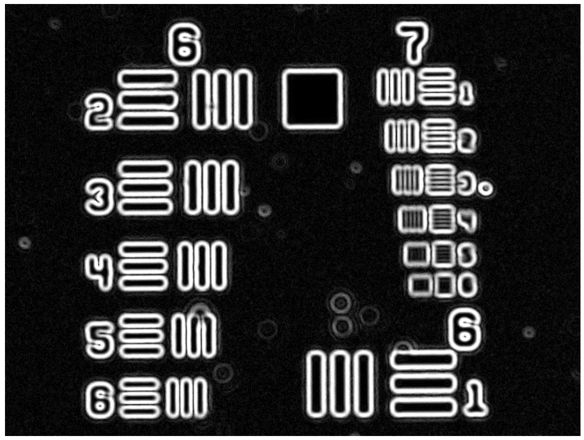

Figure 2. Image taken with a BFP diameter of 7.5mm put through the 'Find Edges' process in ImageJ, which applies an Sobel edge detection.

The Find Edges function uses a different algorithm to that used to form the images in Figure 1. The following convolution kernels are applied to the image separately, and then square root of the sum of the squares are taken from both convolutions.

1	2	1
0	0	0
-1	-2	-1

1	0	-1
2	0	-2
1	0	-1

Despite the different algorithms, Figure 2 is visibly similar to the images in Figure 1. This suggest the image information pertaining to the edges in an image are distributed in the outer radius of the objective BFP. The reason for this is that an edge, being a quick change, could be approximated as a very small lens, which would have a large radius of curvature, this would cause light to scatter into the outer bounds of the lens.

Oblique Illumination

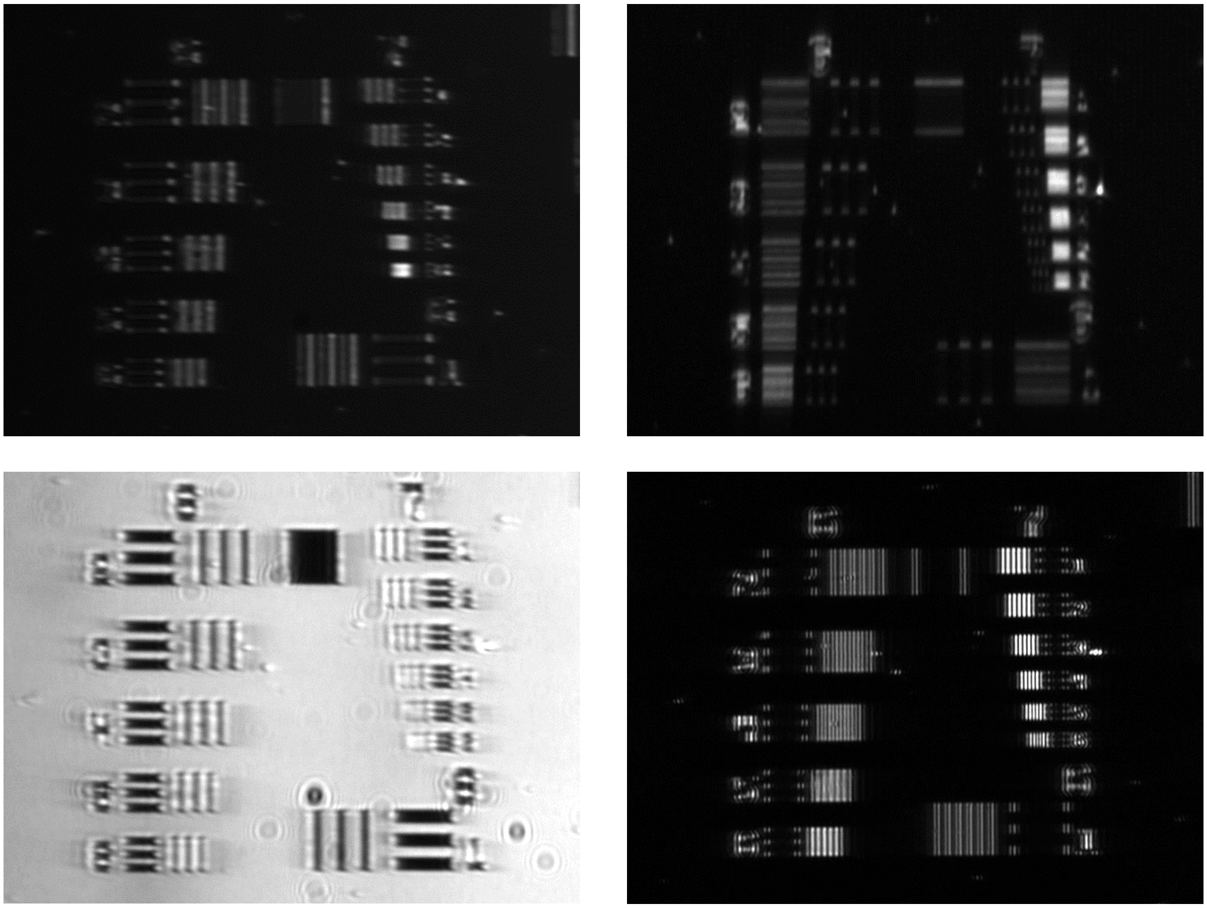

Figure 3 (Top Left): Oblique Illumination with Tin foil orientated vertically. (Top Right): Oblique Illumination with Tin foil orientated horizontally. (Bottom Left): Oblique Illumination with Tin foil orientated horizontally; foil masks are separated slightly such a sliver of light is allowed to pass between. (Bottom Right): Ball driver used to obstruct the center of the image, same as shown in Figure 1.

It can be seen with the Oblique illumination that vertically aligned masks illuminated the vertical line pairs whereas the horizontal masks illuminated the horizontal line pairs. The picture using the ball driver appears most similar to the vertical masks, which is likely caused by the driver being placed vertically in the system also. Acting as a finite rectangle mask, it is similar to and inversion of the vertical masks.

This suggests the information about elements exist in certain planes perpendicular to the objective BFP. Vertical features exist horizontally to pass through a vertical mask in the back focal plane and horizontal features exit vertically to pass through a horizontal mask.

Point Spread Function

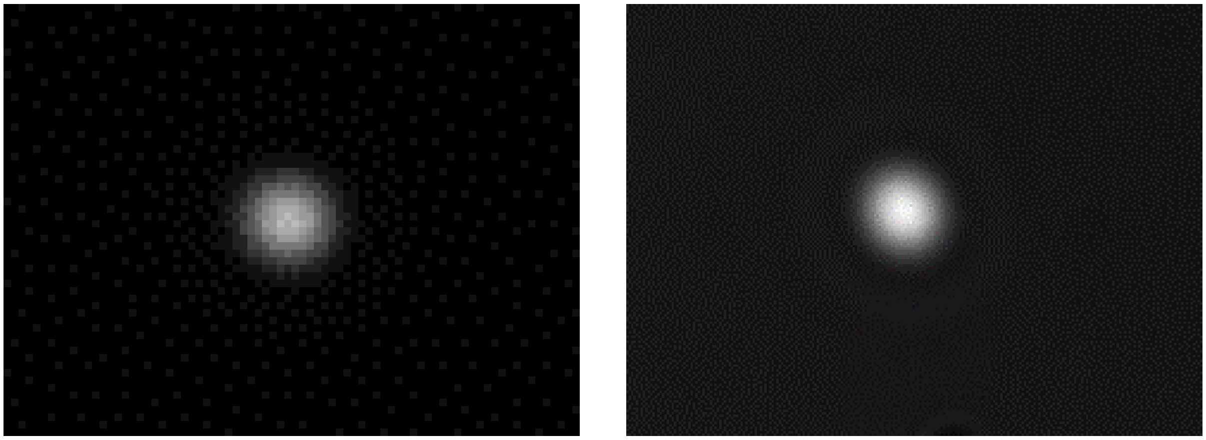

Figure 4. (Left): Image of 5um pinhole with objective BFP of 3mm. Exposure time 4.8ms. Wavelength 528nm. (Right): Image of 5um pinhole with objective BFP of 1mm. Exposure time 288ms. Wavelength 528nm. The first ring around the airy desk can be faintly seen in this image.

Images were dim due to the limited amount of light allowed through the pinhole. As the pinhole is physically very small, almost no light from the illumination makes it through the pinhole. Therefore, the image in not bright, and a large exposure time must be used to make the image visible.

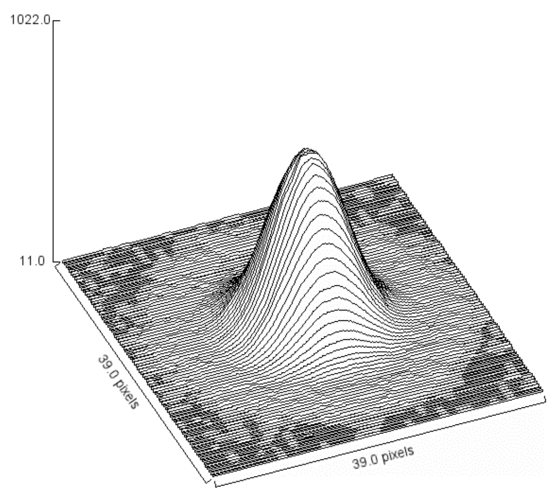

Figure 5. Surface Plot of the 5um pinhole with object BFP of 3mm, seen in Figure 4. Plot shows the Intensity (vertical) of each pixel in the image. The 3d function is not cubic as might be expected if raytracing is done but shows evidence of the wave nature of light. The shape of the surface appears similar to a Gaussian or Bessel function, which is expected for a PSF to occur.

The pinhole with a obj BFP of 3mm has diameter of 22px across. As the camera pixel spacing is 3.45um and the Magnification is 6, the airy disk is thus 12.65um in diameter.

The theoretical airy disk size is twice the Rayleigh criterion, and is thus:

Equation 1 $\sigma = \frac{1.22\lambda}{NA}$

For the objective lens with an BFP diameter of 3mm and a focus of 25mm, the airy disk diameter is expected to be 10.7um; giving an error of 17.8%. Error is caused by the finite sized pinhole, which causes the airy disk to be larger as it is a collection of points versus a true PSF.

The tube lens in the system has a focal length of 150mm, and a diameter of 25mm, However, the light from the BFP only illuminated lens with a diameter of the BFP, which was set to 3mm. This would give a NA of 0.01, and an airy disk diameter of 64um.

If the BFP diameter was decreased to be 1mm, the NA would be 0.003, giving a airy disk diameter of 193um.

For a 1.0mm BFP diameter, the NA would be 0.02, giving a theoretical Airy Disk Diameter of 32um. The actual airy disk is measured as being 34.5um giving a error of 7%.

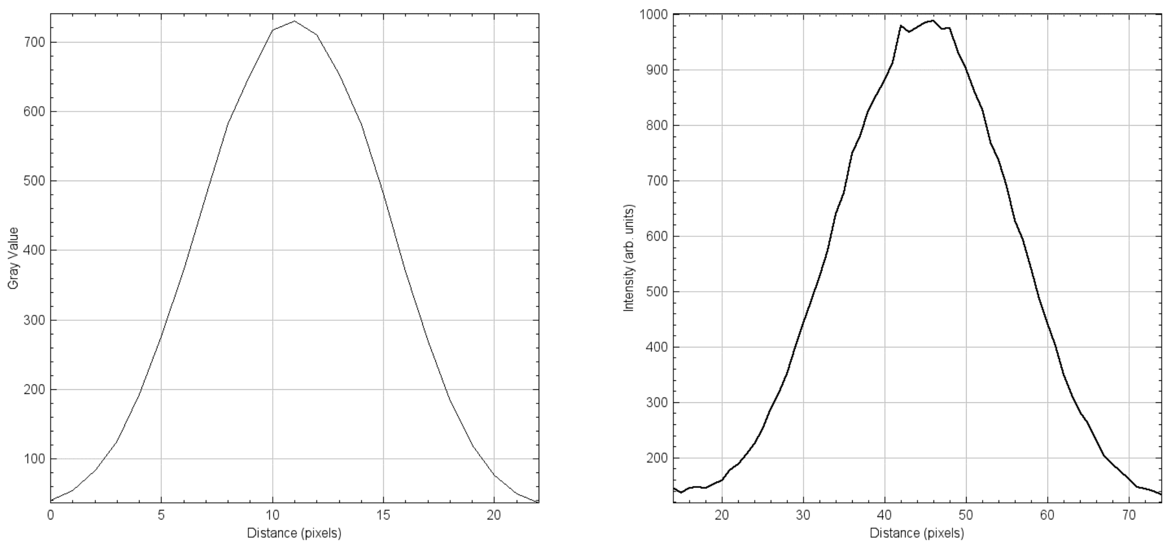

Figure 6. (Left): Line plot of the Airy Disk with Objective BFP = 3mm. (Right): Line plot of Airy Disk with Objective BFP = 1mm. Diameters for each airy disk are listed in Table 1.

Iris diameter (mm)	NA	Airy Disk diameter	actual diameter (px)	actual diameter (um)	error %
3	0.06	10.7	22	12.65	17.8
1	0.02	32.2	60	34.5	7.1

Table 1. Theoretical Airy Disk versus the measured diameter

The ratio between the calculated airy disk of the objective BFP and the airy disk of the tube lens is calculated in Equation 2.

Equation 2: $\frac{Airy\ Disk\ obj}{Airy\ Disk\ tube} = \frac{10.7mm}{64.4mm} = 6$

This is equal to the magnification of the system which can also be calculated used the focal lengths of the tube and objective lens' in Equation 3.

Equation 3: $\frac{f_{tube}}{f_{objective}} = \frac{150mm}{25mm} = 6$

As the airy disk diameter is dependent of the NA, Equation 2 and Equation 3 are shown to be equal by:

Equation 4. $\frac{Airy\ Disk\ obj}{Airy\ Disk\ tube} = \frac{1.22\lambda}{1.22\lambda}\left(\frac{1/NA_{obj}}{1/NA_{tube}}\right) = \frac{1.22\lambda/r}{1.22\lambda/r}\left(\frac{f_{tube}}{f_{objective}}\right) = \frac{f_{tube}}{f_{objective}}$