Lab Write-Up Week 4

Kohler Illumination

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Kohler Illumination

The microscope setup was set up to examine the test sample using Kohler illumination.

In this setup, the image of the lamp is out of focus between the collector lens and the aperture stop, whereas the lamp filament is in focus at the aperture stop.

The light from the lamp looks like a consistent circle of light between the condenser lens and the sample plane. The filament is not in focus at all. This is because, as stated, the filament is in focus at the aperture stop which is a conjugate plane with the lamp. As the aperture stop is places one focal length away from the condenser, the lamp will also be imaged at infinity, so will be completely unfocused between the condenser and the objective lens.

The image of the filament is also visible on the back focal plane of the objective lens. If the tube lens and objective lens are placed in a 4f configuration, then the image of the filament should be completely unfocused at the imaging sensor.

Filter Effects on Resolution

The effect of placing a filter in front of the white Halogen light on resolution was measured. The Halogen Lamp has a peak wavelength of 709nm, and a bandwidth of 278nm at FWHM.

The IR Filter is manufactured with a bandpass region between 335 and 610nm, which cuts out the majority of the wavelengths emitted by the halogen bulb as seen in Figure 1. When used with the Halogen lamp, the IR filter produces a light with a wavelength of 581 nm and FWHM of 115 nm; this is narrower than the halogen light, as the filter lets through a subset of its frequencies.

Using the green filter, the light has a peek wavelength of 536nm, with a FWHM of 64nm. This is both a lower peak wavelength and a narrow bandwidth than using the IR filter, which should be better for imaging.

Using the IR filter and the green filter in combination has practically no effect, with only a difference of 1.5% between the two peeks. This can be expected as the bandpass of the green filter is a subset of the of that of the IR filter, making the IR filter redundant in this system. This can be seen in Figure 1, where the curve of the IR Filter and the IR + Green spectrums visibly overlap each other nearly perfectly.

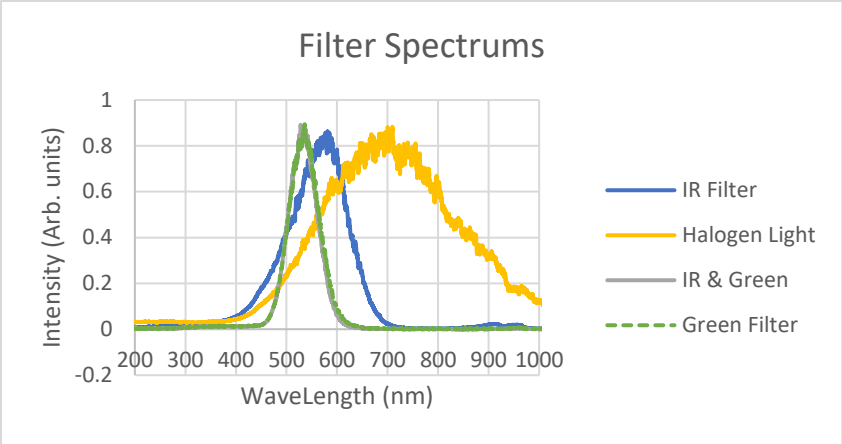

Figure 1. Spectrums of the light with filters applied. Using the filters narrows the wavelength of the light from the outputted by the halogen bulb. Using the Green filter creates the narrowest waveband, and using the IR filter and Green filter has almost no effect compared to just the green filter.

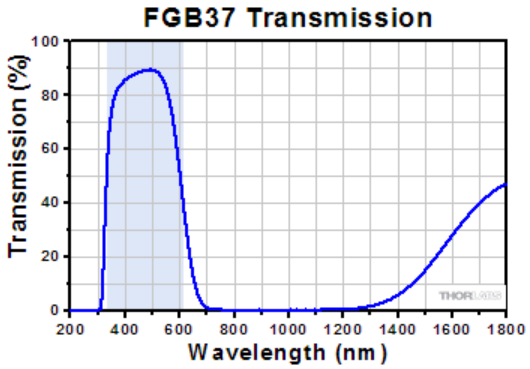

Figure 2. Transmission intensity versus wavelength for the FGB37M IR filter. Rated for 335-610nm. Graph from Thorlabs.

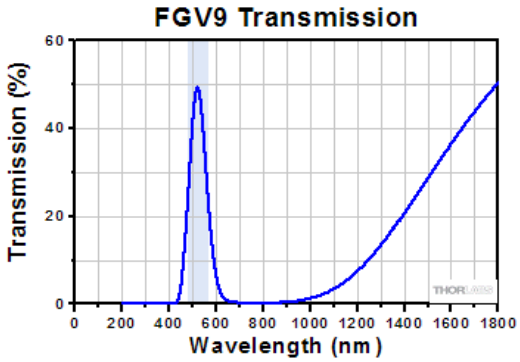

Figure 3. Transmission intensity for a FGV9 Green Color filter similar to the one used (exact model is unknown). Rated for a bandpass between 485-565nm. Graph from Thorlabs.

Filter Type	Peak Wavelength (nm)	FWHM (nm)	USAF Target Smallest Resolvable (Group, Element)	Measured Resolution (um)	NA Objective	NA Condenser	Rayleigh Resolution (um)
No Filter	709	278	G7, E1	7.81	0.06	0.06	7.21
IR Filter	581	115	G7, E3	6.20	0.06	0.06	5.91
Green Filter	536	64	G7, E4	5.52	0.06	0.06	5.45
IR + Green	528	63	G7, E4	5.52	0.06	0.06	5.37

Table 1. Effects of light filters on resolution. The best (minimum) resolution is achieved by both minimizing the peak wavelength as well as the bandwidth of the light. Best resolution was achieved by using the IR and Green filters.

Using the filters has the effect of reducing the bandwidth of the light emitted from the lamp, thus reducing chromatic aberration effects, and increasing resolution.

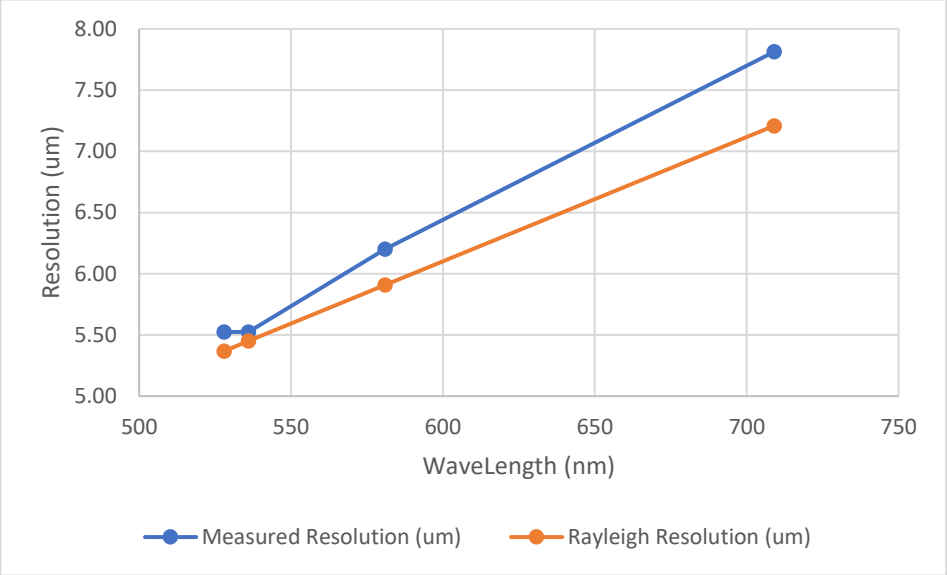

Figure 4. Resolution vs Wavelength, Measured Resolution is worse than the theoretical maximum. Measured resolution is also seen growing at a faster rate than the Rayleigh resolution, due to chromatic aberrations as the waveband increases with wavelength.

The measured resolution and the Rayleigh Resolution both grow together, however, the measured resolution value increases as at a faster rate. This is because the bandwidth of the light is directly correlated to wavelength. An increase in bandwidth increases the effect of chromatic aberration, increasing the minimum resolvable resolution.

Resolution versus Contrast

The resolution test target was imaged using the IR and Green filter; using a BFP diameter of 3mm and aperture stop 3mm in diameter. The Contrast for each test element was measured as:

$$contrast = \frac{max - min}{max + min - 2 * background}$$

The background intensity was measured by taking the average of the pixels inside the black reference square.

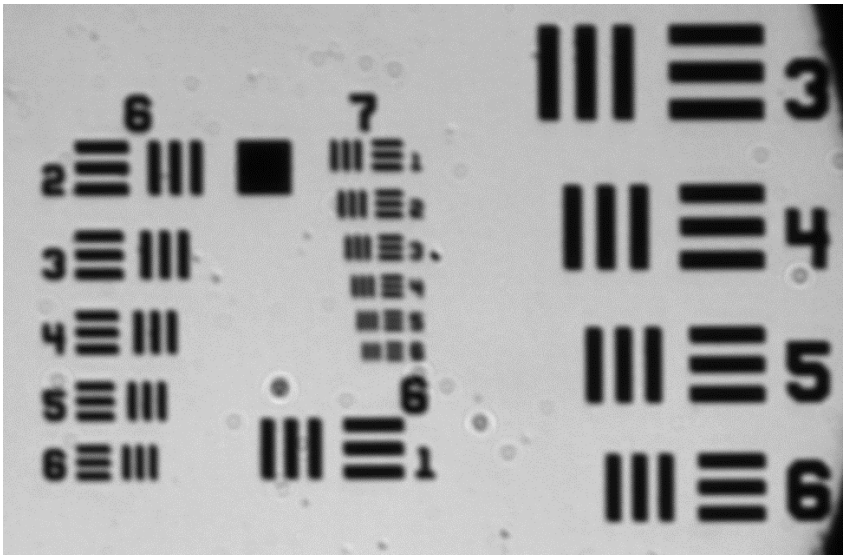

Figure 4. Resolution test imaged using a BFP and aperture stop diameter of 3mm. Using IR, Green filter, and ND1 filter. With an exposure time 5.493ms. Group 5 elements can be seen on the right.

Contrast greatest with the largest elements, and decreases as the elements get smaller, and the line pairs blur together. Contrast vs lp/mm is plotted in Figure 5. Contrast can be seen as being almost constant; remaining above 90% for the first 6 elements, and then decaying linearly until elements are no longer resolvable.

USAF Target Group, Element	USAF lp/mm	Min Pixel Value	Max Pixel Value	Resolution (um)	Contrast %
G5, E4	45	50.8	749	22.1	97.1
G5, E5	51	52.7	764.7	19.7	96.6
G5, E6	57	56	784.8	17.5	95.8
G6, E1	64	63.3	786.4	15.6	94.0
G6, E2	72	65.5	678.7	13.9	92.4
G6, E3	81	72.6	647.7	12.4	89.9
G6, E4	91	82.8	586.1	11.0	85.5
G6, E5	102	101	514	9.8	77.3
G6, E6	114	130.5	454.3	8.8	64.2
G7, E1	128	154	399	7.8	51.8
G7, E2	144	177.9	355.5	7.0	39.2
G7, E3	161	197.9	321.1	6.2	28.1
G7, E4	181	240.05	297.45	5.5	12.6

Table 2. Contrast versus Resolution of elements in Figure 4. Background intensity is measured as being 40.2 by taking the average value inside the black reference square between Groups 6 and 7.

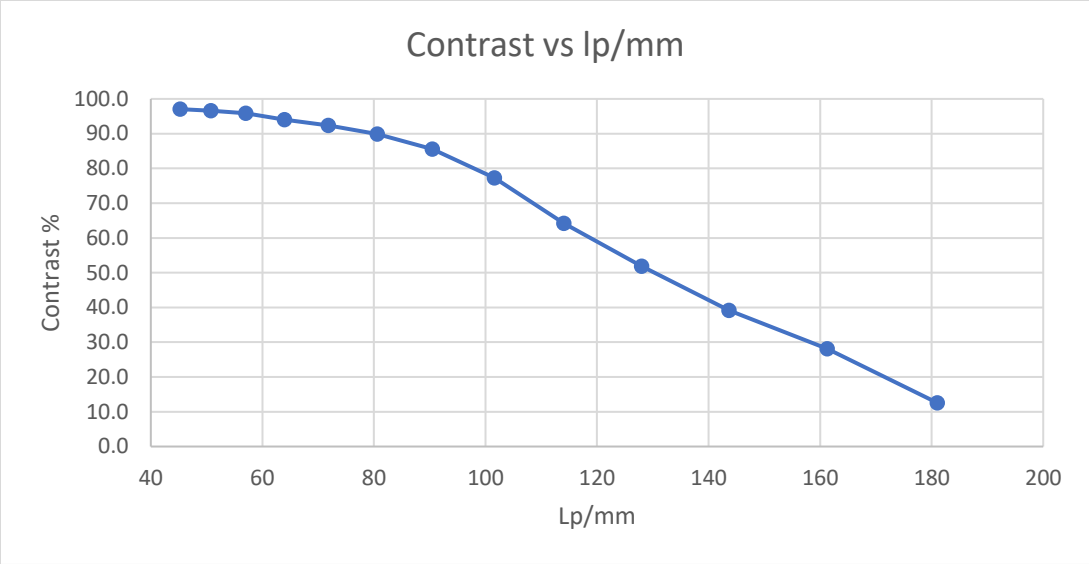

Figure 5. Plot of Contrast versus the lp/mm. Contrast is inversely proportional to linespacing when LP/mm are greater than 90. When Lp/mm are less than 90, the curve flattens, as contrast is logically bounded to be below 100%. This is also expected as when the Elements being imaged are larger (less LP/mm) compared the wavelength of light; then diffraction effects will be less pronounced.

Numerical Aperture on Resolution

The test target was imaged with a BFP iris diameter between 1 and 3.5 mm in steps of 0.5 mm in order to measure the effect of Numerical Aperture on resolution. The smallest resolvable element was found to be the resolution at that iris diameter. Figure 5 shows the minimum resolution viewable at each iris diameter. Resolution is seen improving as the iris diameter increases. Figure 6 shows the relation between the measured resolution and the Rayleigh resolution.

BFP Iris diameter, mm	Objective NA	Rayleigh Resolution (um)	USAF Target Smallest Resolvable Group, Element	USAF Target Max Resolvable ln/mm	Measured Resolution (um)
1	0.02	16.1	G5, E3	40.3	24.8
1.5	0.03	10.7	G6, E3	80.6	12.4
2	0.04	8.1	G6, E6	114.0	8.8
2.5	0.05	6.4	G7, E2	143.7	7.0
3	0.06	5.4	G7, E4	181.0	5.5
3.5	0.07	4.6	G7, E5	203.2	4.9

Table 3. Iris diameter and measured resolution. As the Iris diameter is increased, the resolution of the system also increases.

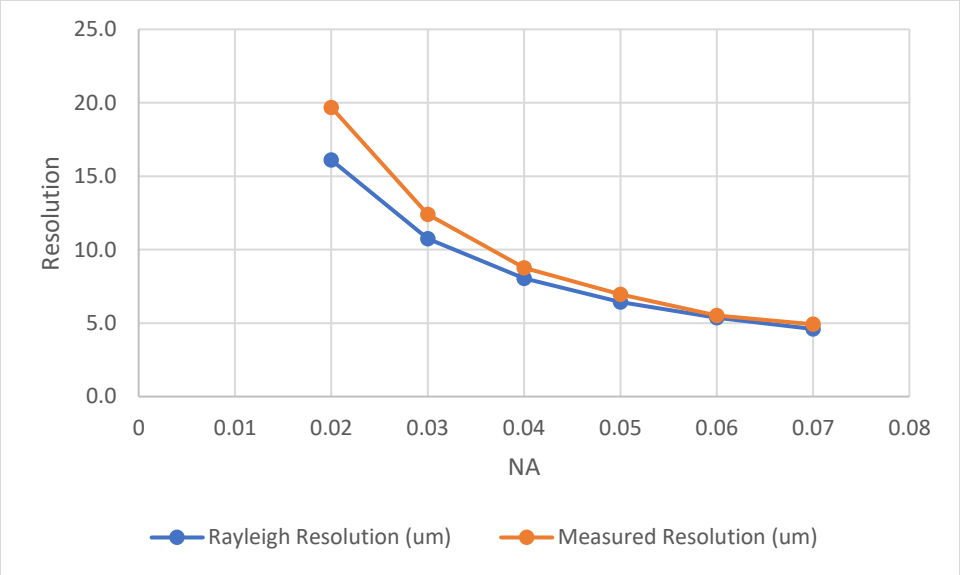

Figure 6. Plot of Resolution vs Numerical Aperture. Measured and Rayleigh Resolution trend the same way.

The measured resolution and Rayleigh Resolution match closely at higher numerical apertures, but as NA approaches 0, the measured resolution gets worse at greater rate than the Rayleigh resolution. This is unexpected as spherical aberration would cause a greater difference at larger NA instead of smaller. Potentially, this may be caused by the construction of the iris, where as small radius the polygonal nature of the iris affects imaging.

Numerical Aperture on Brightness

An area of the test target was imaged, and the pixel intensities were measured to find determine the brightness of the image. The Image was taken with an Iris diameter ranging from 1 to 3.5 mm. Then the average pixel value was measured. Keeping the same exposure, a lens cover was held in front of the filed stop in order to block all of the light from the lamp, and the background intensity of the image was recorded. This background light is generated from other light sources in the lab, that enter the optical system and should be removed as a source of bias in our measurements.

BFP Iris diameter, mm	Calculated NA	Pixel Avg	Background Avg	exposure (ms)	Brightness
1	0.02	680	66	39.413	15.6
1.5	0.03	670	27	17.192	37.4
2	0.04	670	19	11.304	57.6
2.5	0.05	675	12.5	7.208	91.9
3	0.06	707	10	5.8	120.2
3.5	0.07	722	9	4.904	145.4

Table 4. Iris diameter and image brightness. Brightness increases as the iris diameter increases.

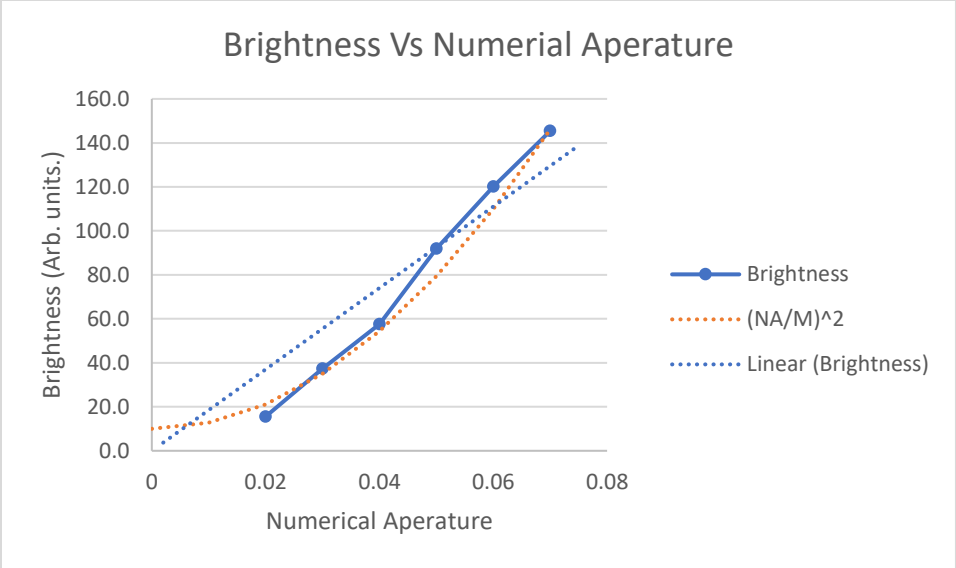

Figure 7. Brightness vs Numerical aperture. The brightness curve is evidently not linear but rather follows the curve (NA/m)^2 where M = 6 times a factor of 10^-3.

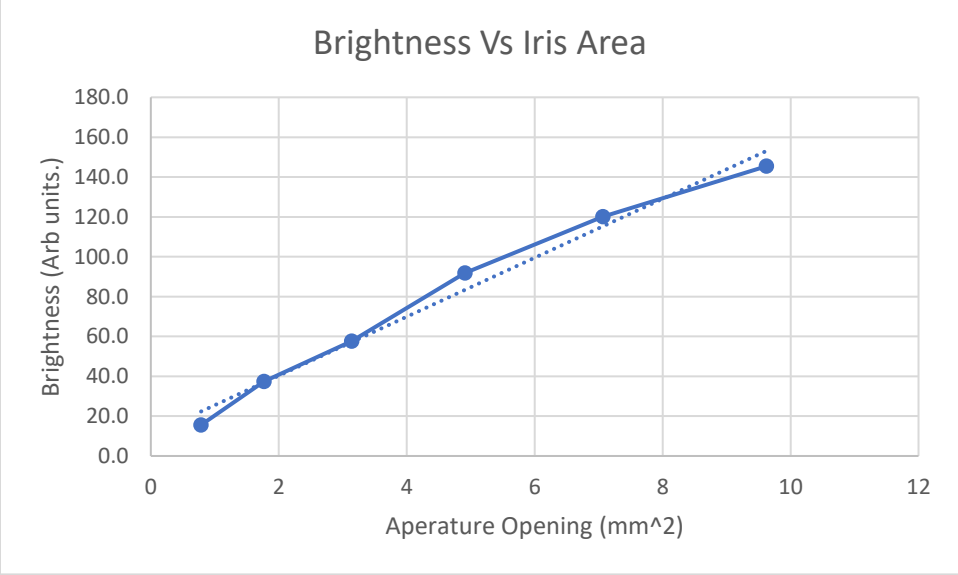

Figure 8. Brightness vs Iris area. The area of the opening is = $\pi \left(\frac{d}{2}\right)^2$ The correlation between the opening of the aperture and brightness is linear as expected.

Numerical Aperture squared is correlated directly with brightness, and iris area is linear with brightness. This is expected as the more light that is let through the iris the brighter the image will be. As Kohler illumination ensures that the light is constant across the image, area and light are directly related.