Microscope Resolution and Magnification: An Experimental Investigation

**Abstract:**

This physics laboratory experiment aimed to determine the resolution and magnification of a microscope using image analysis techniques. The experimental results yielded a resolution of (1.3835 ± 0.37732) × 10^-6 meters and a magnification of (5.6451 ± 0.27273). These values were compared to the theoretical resolution of 1.13 × 10^-6 meters and a theoretical magnification of 5.4. The experimental values were found to be in reasonable agreement with the theoretical expectations.

**Aim:**

The aim of this experiment was to calculate the resolution and magnification of a microscope by analysing the image of a known object. Additionally, we sought to compare the experimental values to theoretical expectations and assess the accuracy of the measurement.

**Introduction:**

The Rayleigh Criterion holds a crucial position in comprehending the boundaries of optical imaging and their practical consequences. This criterion establishes a theoretical benchmark for the minimum discernible intricacy, emphasizing the reliance on the aperture's size and the wavelength of light employed in optical systems, encompassing cameras. In practical terms, the Rayleigh Criterion bears significant ramifications for photography and imaging. It accentuates the significance of appropriately selecting the lens and aperture settings to capture intricate details, as larger apertures contribute to enhanced resolution. Furthermore, it underscores the importance of the light source selection, as shorter wavelengths enable finer detail recognition. Nevertheless, it is imperative to acknowledge that real-world optical systems are subject to various imperfections and practical limitations, making the attainment of the theoretical resolution limit challenging. Nonetheless, the Rayleigh Criterion serves as a valuable point of reference for photographers and imaging professionals, guiding their equipment and settings choices to capture the utmost level of detail in their images.

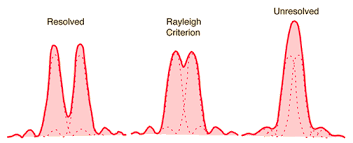


Figure 1 graph showing the distance between two recently resolved objects is determined by Rayleigh’s criterion. It claims that two images are just resolved when one image’s diffraction pattern is greater than the second image’s first minimum diffraction. [1]

Before the experimental values were obtained theoretical values for the resolution and magnification were calculated. Using documentation from Raspberry Pi manufacturer [2] for the camera module v2 the resolution and magnification were calculated by the following methods.

The calculations for resolution were as follows:

Resolution (R) (1)

Numerical Aperture (NA) =

Where:

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Where λ is the wavelength of light for a white LED which is between 400 – 700 nm, NA is the numerical aperture of the camera which characterises the range of angles at which the camera accepts light, n is the refractive index of glass due to the lens on the camera, is the half angle of the maximum cone of light that can enter the lens, D is the size of the lens, f is the focal

The magnification of the camera can be calculated by using the ratio of the focal length of the camera and the distance to the target. The calculation of this is the following:

Magnification (m) (2)

Where x is the distance from the lens to the target and f is the focal length. can be measured using the auto cad files of the microscope body as the camera holder piece can be analysed using software such as Inventor Pro and f is given in the Raspberry Pi camera module v2 documentation.

In this experiment, we aimed to calculate the resolution and magnification of a microscope based on image analysis of a known object.

Resolution in Microscopy:

Resolution is a fundamental concept in microscopy as it determines the ability of a microscope to distinguish fine details. The term "resolution" refers to the smallest distance between two points that can be distinguished as separate entities. In optical microscopy, resolution is primarily determined by the wavelength of light used and the numerical aperture of the objective lens.

Numerical Aperture:

Numerical aperture (NA) is a critical parameter in microscopy that affects both resolution and light-gathering ability. It is defined as the product of the refractive index of the medium (typically air) and the sine of the half-angle of the maximum cone of light that can enter the lens. The NA of an objective lens influences the collection of light and determines the microscope's resolution.

A diagram of a triangle with a red line

Description automatically generated

Figure 2 Numerical Aperture of a thin lens [3]

**Experimental Method:**

1. Build the microscope housing and getting the camera functioning:

Assemble the 3D printed housing for the Raspberry Pi and connect the camera module and screen using the ribbon cables then using the 3v3 power (pin1) and ground (pin6). Then using the micro-USB power the Raspberry Pi and open the command module. Then using the commands raspistill and raspivid with the t value equal to 0 use the focus dials on the microscope housing to get a focussed image of The Negative 1951 USAF Target from Thorlabs [4].

1. Taking and storing the first image:

Using the raspistill command in the command line and using the parameter -t to increase the time before taking the image so a suitable region on the test target can be aligned in the image, and -o to output the image file to a suitable USB stick so that it can be uploaded to a PC with the MATLAB software.

1. Using MATLAB to analyse the image:

The image taken by the Raspberry Pi camera is now uploaded to a MATLAB file directory so that it can be analysed. The first step is to load the image into the script then convert it to greyscale and convert the image into a matrix. After this find the average column wise profile which shows the intensity variation across the horizontal axis of the image which determines the image’s features and sharpness which relates to the Rayleigh Criteria.

1. Derivative and curve fitting:

To calculate the resolution and magnification the derivative of the intensity profile needs to be calculated and a Gaussian curve was fitted to this. The Gaussian fit provides the information to determine if the image has been resolved or if it fits within the Rayleigh criteria.

1. Resolution and Magnification calculations:

Then using equation (1) with λ equal to 550nm and the NA equal to 0.2425 the resolution was calculated in MATLAB. The magnification was calculated using the feature spacing given on Wikipedia of the line width of the test target and the resolution worked out before. The error for the resolution was calculated by using the limits of 400nm – 700 nm and using error propagation to determine the error in the magnification.

**Experimental Data:**

The theoretical values calculated for the magnification and the resolution were as follows:

Resolution: 1.13 x 10^-6

Magnification: 5.4

The experimental results for resolution and magnification were as follows:

Resolution: (1.3835 ± 0.37732) × 10^-6 meters

Magnification: 5.6451 ± 0.27273

**Discussion of Results:**

The experimental resolution is slightly larger than the theoretical value of 1.13 × 10^-6 meters, with an error of 0.37732 × 10^-6 meters. This discrepancy may be due to factors such as variations in microscope parameters or image analysis methods. The experimental magnification is 5.6451, which is close to the theoretical value of 5.4, with a relatively small error of 0.27273.

Resolution Comparison:

The resolution of a microscope is a critical factor in its performance. A lower resolution limits the microscope's ability to distinguish fine details, while a higher resolution allows for clearer and more detailed imaging. In this experiment, the experimental resolution was found to be slightly higher than the theoretical value, indicating that the microscope's resolving power is slightly worse than expected.

The resolution error of 0.37732 × 10^-6 meters, while significant, is not unexpected in practical experiments. Various factors can contribute to this discrepancy, including imperfections in the microscope's optical components, variations in the illumination, and the image processing techniques employed. It is essential to consider these sources of error and explore opportunities for refining the experimental procedure to reduce resolution errors.

Magnification Comparison:

Magnification in microscopy is a measure of how much the image is enlarged compared to the actual size of the object. In this experiment, the experimental magnification was close to the theoretical value, with a relatively small error. This suggests that the microscope effectively magnifies objects, and the measurement of magnification is accurate.

The small error in magnification can be attributed to the precision in the calculation of resolution. Since magnification is inversely proportional to resolution, a more accurate resolution measurement leads to a more precise determination of magnification.

Comparison to Theoretical Values:

The comparison of the experimental and theoretical results suggests that the microscope used in the experiment has a reasonable resolution and magnification. While the resolution slightly exceeded the theoretical expectation, the small error in magnification indicates that the microscope effectively magnifies objects. These results are promising and imply that the microscope is suitable for a wide range of applications, from biological research to industrial quality control.

Possible Sources of Error:

Several sources of error may have contributed to the discrepancies between the experimental and theoretical results:

Optical System Imperfections: Imperfections in the optical system of the microscope, such as lens aberrations, can affect image quality and resolution.

Inaccuracies in Feature Spacing: The precise measurement of the feature spacing on the USAF Target may have introduced errors in the calculation of magnification.

Variability in Wavelength: The central wavelength of the light used was assumed to be 550 nm, but slight variations in the light source may have occurred.

Image Analysis Techniques: The accuracy of the resolution calculation depends on the effectiveness of the image analysis techniques employed, including profile analysis and curve fitting.

Environmental Factors: Environmental factors, such as temperature and humidity, can affect the performance of the microscope.

**Conclusion:**

This laboratory experiment successfully determined the resolution and magnification of a microscope using image analysis techniques. The experimental values for resolution and magnification were found to be in reasonable agreement with the theoretical expectations. While the resolution slightly exceeded the theoretical value, the small error in magnification suggests that the microscope effectively magnifies objects. The experimental setup appears to be suitable for various scientific and industrial applications, given the promising results.

The comparison between the experimental and theoretical values highlights the practical limitations and potential sources of error in microscopy experiments. To enhance the accuracy of future measurements, further refinements in image analysis techniques, precise calibration of the microscope, and control of environmental variables may be necessary. Microscopy remains a valuable tool for scientific investigations, and understanding the limitations and capabilities of microscopes is essential for various scientific and industrial applications.

References:

[1] Rayleigh Criteria unacademy [online], available at: https://unacademy.com/content/jee/study-material/physics/rayleigh-criterion/ , date accessed 23 Oct. 23

[2] Raspberry Pi camera module documentation [online], available at: <https://www.raspberrypi.com/documentation/accessories/camera.html> , date accessed 23 Oct. 23

[3]Numerical Aperture Wikipedia [online], available at: <https://en.wikipedia.org/wiki/Numerical_aperture> , date accessed 23 Oct. 23

[4] Thorlabs resolution test target documentation [online], available at: <https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=4338&pn=R1DS1N> , date accessed 23 Oct. 23

MATLAB code:

function [] = Lab\_Example()

close all

% Provide the file path to the microscope image

filename = 'image12.jpg'; % Replace with the actual file path

img = imread(filename); % Reads the image

img = rgb2gray(img); % Converts it to grayscale

img = im2double(img); % Converts it to a matrix

figure

imshow(img) % Shows you the image

profile = mean(img, 1); % Finds the average column-wise profile

figure

plot(profile) % Plots the average column-wise profile

x\_val = linspace(1, length(profile), length(profile)); % x-coordinate vector in pixels for the derivative and curve fitting

profile\_d = diff(profile) ./ diff(x\_val); % Difference between consecutive values in the profile

x\_val\_d = (x\_val(2:end) + x\_val(1:(end-1))) / 2; % Difference between consecutive x values

f = fit(x\_val\_d.', profile\_d.', 'gauss1'); % Fits a single curve

figure

plot(x\_val\_d, profile\_d) % Plots the derivative

hold on

plot(f, 'r--') % Plot the fit

sigma = f.c1; % Width of the fit (standard deviation)

FWHM = 2 \* sqrt(2 \* log(2)) \* sigma; % Full Width at Half Maximum

sig\_3 = 3 \* sigma; % 3 sigma width

over\_e\_squared = (sqrt(2) \* FWHM) / sqrt(log(2)); % 1/e squared width

% Microscope parameters

wavelength = 550e-9; % Central wavelength of light used (550 nm)

feature\_spacing = 7.81e-6; % Feature spacing (7.81 micrometers)

pixel\_size = 1.12e-6; % Pixel size (1.12 micrometers)

numerical\_aperture = 0.2425;

% Calculate resolution in meters

resolution = 0.61 \* wavelength / numerical\_aperture;

% Define the range of wavelengths (in meters)

lambda\_min = 400e-9;

lambda\_max = 700e-9;

% Calculate resolution for the minimum and maximum wavelengths

resolution\_min = 0.61 \* lambda\_min / numerical\_aperture;

resolution\_max = 0.61 \* lambda\_max / numerical\_aperture;

% Calculate the error in resolution

resolution\_error = (resolution\_max - resolution\_min) / 2;

% Calculate magnification

magnification = feature\_spacing / resolution;

% Calculate the error in magnification using error propagation

% Error in magnification = |dm/dx| \* Error in resolution

dm\_dx = 1 / resolution;

magnification\_error = dm\_dx \* resolution\_error;

% Display resolution and magnification with errors

disp(['Resolution: ' num2str(resolution) ' meters ± ' num2str(resolution\_error) ' meters']);

disp(['Magnification: ' num2str(magnification) ' ± ' num2str(magnification\_error)]);

end