

# Title of the document

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## Abstract

The report starts with a Samenvatting (Abstract). The abstract should be self-contained, i.e. a reader should be able to fully understand it without any prior knowledge about the research. Also, in the abstract there should be no references to (figures, tables, formulas etc. in) the remainder of the report, nor to the literature. The abstract tells the reader:

- (1) which research question has been studied,
- (2) what the research method/approach was,
- (3) which results have been obtained, and
- (4) what the main conclusions were.

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# 1 List of symbols

bla bla bla

bbb

## 2 Introduction

Microscopes are used extensively in natural sciences. They enable us to image small objects and structures which cannot be resolved by the human eye. The use of microscopes, could for example, aid in studies of biological cells, molecular structures or object classification. To correctly conduct such microscopy studies, it is vital to know what the possibilities and limits of the particular microscope are in combination with image improvement techniques.

This experiment will focus on a Leica DM EP polarising microscope in combination with a colour CCD and to what extent this set-up can be used to measure the size of small objects and find the birefringence of an unknown crystal. Furthermore, the possibilities of digital image improvement will be investigated.

After calibrating the pixels and finding the resolving power with the aid of a microscopic ruler and resolution target respectively. Images are made of a human hair, an optical fiber, starch particles, an unknown birefringent crystal and a fungus sample.

To find the size of the human hair, particles and fiber computer techniques will be used. The birefringence of the crystal will be determined by focussing on the differently coloured layers of the crystal, this way we can determine the thickness of each layer and its colour to subsequently calculate the birefringence. Finally some python algorithms are implemented on the image of the fungus to investigate improvements on contrast and colour corrections

In section 2 the theory regarding the experiment will be described, followed by the experimental method in section 3. The results and discussion can be found in section 4. Lastly the conclusions in section 5.

### 3 Theory

In the Theorie (Theory) chapter, you describe all (and only!) the theory needed to understand and interpret the experiments in the remainder of the report. Explain to the reader why a piece of theory is relevant for your research. Equations should be numbered. If an equation cannot be assumed to be generally known by the readers (see General Hint 2 for the level of the audience), you should provide a reference to an accessible textbook or article (so not to the RP manual, lecture notes, Wikipedia etc.). In general, try to avoid referring to websites, online data or Wikipedia.

## 4 Experimental method

This experiment consists of four parts. First the calibration of the microscope. Secondly size measurements on respectively a human hair, an optical glass fibre and starch particles. Thirdly determining the birefringence of an unknown crystal and finally the computerized improvement of an image of a biological fungus sample. The different experimental methods will be treated separately.

The microscope that is used in all experiments is a Leica DM EP microscope. Its manual can be found in appendix ???. This microscope is used in combination with 4 $\times$ , 10 $\times$ , 40 $\times$  Hi Plan POL objectives with respectfully a 0.10, 0.22 and 0.65 numerical aperture. A color ccd camera in combination with NI Vision Assistant software is used to acquire digital images.

### 4.1 Calibration

A microscopic ruler is used to measure the length that corresponds to one pixel in an image. This is achieved by focussing on a 1 mm, 100 division ruler and measuring the distance between two focussed, distant division lines and comparing the number of pixels to the physical length. The NI software is used to find the exact location of these two lines and subsequently find the perpendicular projection (see Figure ??). This procedure is repeated for all three objectives. The relative magnification between two objectives,  $M_{A,B}$  can subsequently be found using equation ??.

With the aid of a 1951 USAF resolution target, the resolving power of each objective can be found. First taking a focussed grayscale image on the target and then taking a perpendicular intensity profile for each well defined three-bar structure (see Figure 4). This intensity profile is exported by NI Vision to a CSV file directly so we don't suffer from any compression, a file which can be easily read by a python peakfinding script ( an example of which can be seen in the next section's figure 5). The high and low intensity values will be used to calculate the visibility using equation ?? and the corresponding spatial frequency. This is repeated for all three objectives.

### 4.2 Microscopic size measurements

All microscopic size measurements are made by measuring pixels and comparing this to the corresponding pixel length. This is done for images with the 40 $\times$  objective since this gives the smallest error.

#### 4.2.1 Human hair and optical glass fibre

Measuring the thickness of the human hair and optical glass fibre is done with the aid of the NI Vision software. First finding the two straight lines of the outer edges and subsequently measuring the perpendicular distance between the two. The actual size and error can be calculated using respectfully equation ?? and ??.

#### 4.2.2 Starch particles

In order to measure the size of individual starch particles, a small amount of starch is mixed with oil. Images are taken at different locations in the mixture. For ellipse-shaped particles that are focussed in the image, an ellipse can manually be fitted.

For this experiment it was chosen to find the ellipse size for 30 particles.

### 4.3 Birefringence

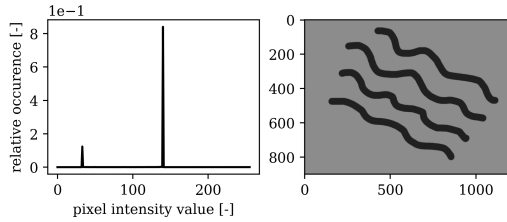
In order to find the birefringence,  $\delta n$ , of the unknown crystal, it is placed in the microscope with the polariser crossed with respect to the analyser. The crystal is then turned until bright colours can be seen. Now the path difference,  $\delta l$ , is measured as a function of the thickness,  $D$ , of the crystal.  $D$  can be found by viewing a border between adjacent colour planes and subsequently noting the focussing position,  $f$ , of each colour plane. Taking the difference between two values of  $f$  will give the difference in thickness,  $d$ , between two colour planes. The bottom of the sample (black) is also to be taken into account with the same procedure as described above.

For this experiment it was chosen to find  $D$  and  $\delta l$  for 5 colour planes. The focussing process was repeated 4 to 5 times for every border that was studied.

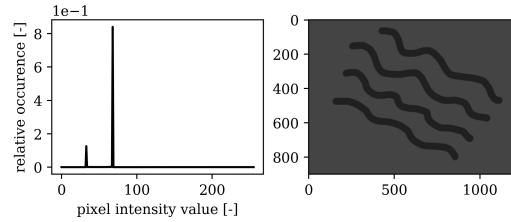
### 4.4 Image improvement methods

The features of an image are easier to distinguish when the image has plenty of contrast, an underexposed image might hold a lot of information that is not yet visible for us.

A way of quantitatively expressing the contrast of an image is through a histogram as outlined in the NI Vision article [1]. To create such a histogram a python script has been written. Two example files were worked on by the python file, the result of which is plotted in figures 2 and 1. Both photos have the same pattern on top but a different shade of grey as a background.



**Figure 1:** High contrast photo



**Figure 2:** Low contrast photo

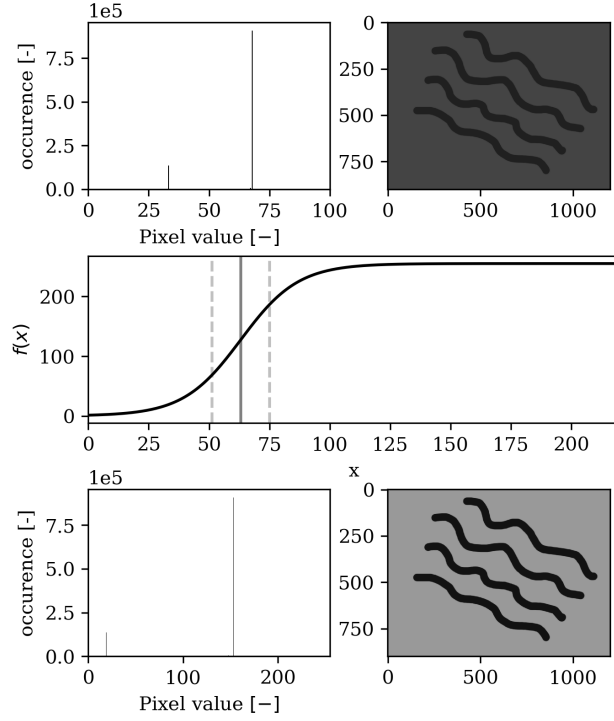
As can be seen in figure 1 the image has high contrast, the peaks from the squigly lines and the background are separated in the histogram. Figure 2 has its peaks closer together meaning that its contrast is lower and thus its features are harder to differentiate from the background.

Our goal in this practicum is to devise a way in which we can alter the contrast of a low contrast photo in such a way that it will be easier to distinguish certain features. This method has its drawbacks however since we artificially widen the gap between the peaks which will mean that while we stretch the information between the peaks over a greater intensity range we compress features in the high and low intensity value range.

One way of widening the gap between the peaks in the histogram is by applying a sigmoid function on the photo, as sigmoid function is a function  $f(x)$  that maps its domain to values between zero and one. This sigmoid equation 1 depends on two parameters  $\alpha$  and  $\beta$  which respectively define the center and the width of the function.

$$f(x) = \frac{1}{1 + e^{-\frac{x-\alpha}{\beta}}} \quad (1)$$





**Figure 3:** The example figure modified using a sigmoid function.

In figure 3 on the left the low contrast example figure and its histogram are plotted in the top row, in the middle row a sigmoid function has been plotted as well as the median value and the standard deviation of the histogram above denoted by the grey solid and dashed line respectively. The bottom row consists of the histogram of the example image and the image acted on by the sigmoid function. In this example we used a sigmoid function  $f'(x) = f(x) \cdot 255$  since we want to map our input values on a domain  $[0, 255]$  not  $[0, 1]$ ,  $\alpha$  is the median of the top histogram and  $\beta$  its standard deviation.

As can be seen in the figure the peaks of the most common values of the intensity have been spread further apart thus resulting in a higher contrast image.

## 5 Results and discussion

### 5.1 Calibration

The values that have been found for  $l_{real}$ ,  $n$ ,  $l_{pixel}$  and the corresponding error are presented in table ?? for each objective. It was estimated that  $u(n) = 4$ .

The images corresponding to each measurement are presented in appendix ??, ?? and ??.

**Table 1:** Results of measurements of  $n$  for the corresponding value of  $l_{real}$  for each objective. The values for  $l_{pixel}$  and  $u(l_{pixel})$  follow from respectfully equation ?? and ??.

Objective	$l_{real}(m) \cdot 10^{-3}$	$n$	$l_{pixel}$	$u(l_{pixel})$
4×	1	$6.85 \cdot 10^2$	$1.461 \cdot 10^{-6}$	$9 \cdot 10^{-9}$
10×	0.8	$1.247 \cdot 10^3$	$6.42 \cdot 10^{-7}$	$2 \cdot 10^{-9}$
40×	0.2	$1.250 \cdot 10^3$	$1.600 \cdot 10^{-7}$	$5 \cdot 10^{-10}$

The values that have been found for  $M_{A,B}$  for the different objective combinations are presented in table ??.

**Table 2:** Calculated values for  $M_{A,B}$  for the three different objective combinations.

Objective A	Objective B	B/A	$M_{A,B}$	$u(M_{A,B})$
×4	×10	2.5	2.28	$2 \cdot 10^{-2}$
×4	×40	10	9.13	$6 \cdot 10^{-2}$
×10	×40	4	4.01	$2 \cdot 10^{-2}$

As expected, the accuracy for the higher magnification objectives is better. Meaning that images from an objective with a higher magnification, corresponds with a smaller value for  $l_{pixel}$ . However, not all the values of  $M_{A,B}$  match the theoretical relative magnification of  $\frac{B}{A}$  taking into account the error.

since only the values of  $M_{A,B}$  with  $B = 40$  do not match the theoretical relative magnification, it would suggest that only the ×40 objective is not true to its specifications. More research could be done to prove this with smaller uncertainty.

Apart from the fact that the objectives might not be true to specifications, this does not affect the results of this experiment. It proves, however, the importance of calibrating a microscopy set-up in order to get valid size measurements.

The values that have been found for  $I_{max}$ ,  $I_{min}$ ,  $V_{is}$  and the corresponding errors are presented in table ?? in appendix ?. In figure ??,  $V_{is}$  is plotted as a function of the spatial frequency for each objective. The images corresponding the measurements are presented in appendix ??, ?? and ??.

### 5.2 Size measurements

The values that have been found for  $d_{hair}$  and  $d_{gf}$  are respectively  $d_{hair} =$  and  $d_{gf} =$ .

The images used for this part of the experiment and the values that have been found for  $a$ ,  $b$ ,  $A$  and the corresponding errors can be in appendix ?. A histogram of the values for  $A$  is presented in figure ??.

### 5.3 Birefringence

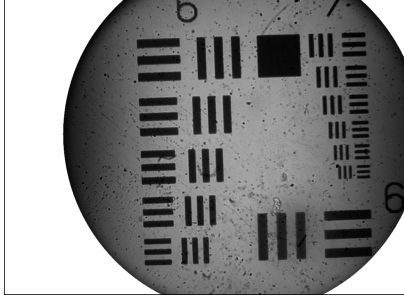
The colour planes that were taken into account for this experiment can be seen in figure ?? in which each Roman numeral corresponds to a colour plane.

The values that have been found for  $D$ ,  $\delta d$  and the corresponding errors are presented in table ?? in appendix ?.

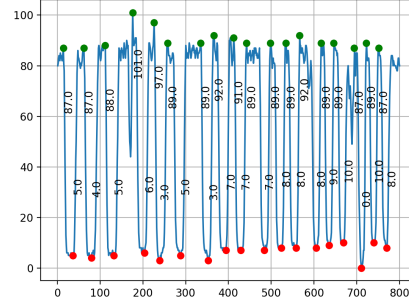
In figure ??,  $\delta d$  is plotted as a function of  $D$ .

It follows from the orthogonal distance regression that  $\delta n = XXX$ .

## 5.4 Resolving power

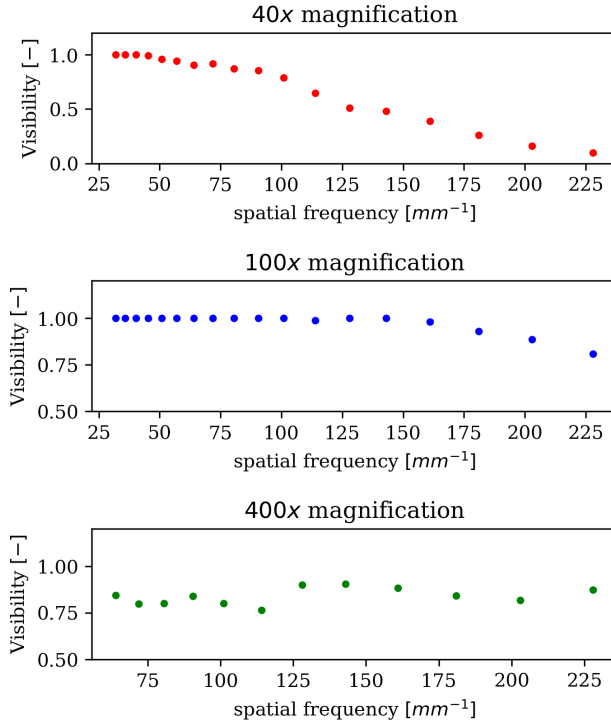


**Figure 4:** Black and white photo.



**Figure 5:** Linetrace of seventh group.

The above photos are the result of the process outlined in the previous section. The high and low values of the line trace were manually read of the photos and entered into a python script capable of calculating the visibility values for each magnification and spatial frequency. The result of which can be seen in figure 6.



**Figure 6:** Plots of the visibilities per numerical aperture.

its photo is evidently less bright than that of the other two apertures. This can be seen when taking a look at either the linetraces or the photos in the appendix.

The data is plotted in such a way that the highest subplot has the lowest magnification and the lowest subplot has the highest magnification. Each subplot has the dimensionless visibility number plotted on the vertical axis and the spatial frequency plotted on the horizontal axis. We chose this layout since we expect the visibility to decrease when the lines get closer together and the spatial frequency thusly increases. Note that only the vertical visibility axis of the highest subplot starts with a visibility of zero.

What we see is not surprising when we also take into account the photos in the appendix. As can be seen on these photos the highest magnification lens has the smallest numerical aperture, therefore all three traced groups are clearly resolvable. Thus the visibility won't drop as much as the lowest magnification lens when the spatial frequency increases.

Something noticeable however is that the highest magnification plot starts off with the lowest visibility value. This has to do with the fact that this smaller aperture also catches less light, the brightest spot in

## 6 Conclusions

In the Conclusions (Conclusions) chapter

- You give a clear and concise answer to the research question that was formulated in the Introduction
- You discuss to what extent, and why, your findings do (not) agree with theory/expectations/earlier work, you discuss more speculative conclusions, and you may do suggestions for further (improved/extended) research. The Conclusions should be self-contained and understandable for readers that have only read the introduction (and have not read the rest of your report, do not know the literature, do not know the experimental setup and have not read the RP manual). In the Conclusions chapter, you may not make references to graphs, tables, equations etc. in the remainder of the report.

## References

- [1] “Image processing with ni vision development module.” <https://www.ni.com/nl-nl/innovations/white-papers/06/image-processing-with-ni-vision-development-module.html>, mar 2019.