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.

Contents

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 polirising 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 unkown 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 har, 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 theTheorie (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×, 10×, 40× 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 **??**. The visibility can subsequently be calculated with 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× 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, δn, 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, δl, 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 δl for 5 colour planes. The focussing process was repeated 4 to 5 times for every border that was studied.

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 1 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 2.

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, δd and the corresponding errors are presented in table **??** in appendix **??**.

In figure **??**, δd is plotted as a function of D.

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

5.4 Resolving power

The photos of the resolution target (of which figure 1 is a example) were shot with the NI Vision software present on the computer we used, using this software we were able to directly create datasets for the linetraces over several groups. This meant that we had no extra artifacts from compression of the photo files. The data was exported as a comma-seperated data file. After trimming the data we used a simple python program to parse the data. The resulting figures were easily readable. One of these figures is shown below in figure 2

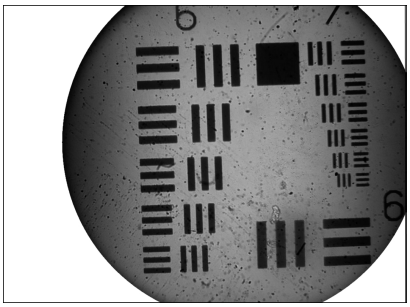

Figure 1: Black and white photo.

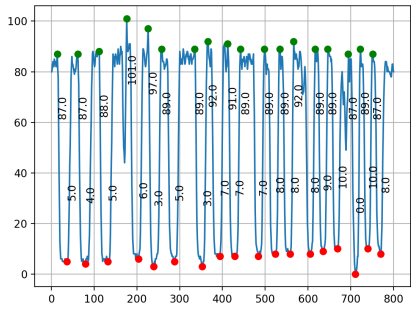

Figure 2: Linetrace of seventh group.

The high an 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 3.

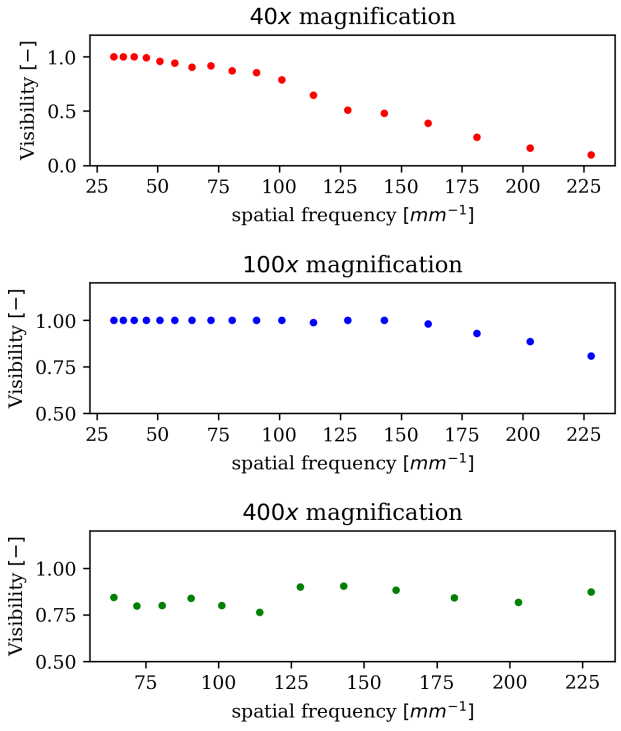

Figure 3: Plots of the visibilities per numerical aperature.

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 verticle 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 lense has the smallest numerical aperature, therefore all three traced groups are clearly resolvable. Thus the visibility won't drop as much as the lowest magnification lense when the spatial frequency increases.

Something noticable however is that the highest magnification plot starts of with the lowest visibility value. This has to do with the fact that this smaller aperature aslo catches less light, the brightest spot in its photo is evidently less bright than that of the other two aperatures. This can be seen when taking a look at either the linetraces or the photos in the appendix.

6 Conclusions

In theConclusies (Conclusions) chapter
-You give a clear and concise answer to the research question that was formulated in the Intrduction
-You discuss to what extent, and why, your findings do (not) agree with theory/expectations/earlier work, you discuss more speculative conclusions, ad 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 graps, tables, equations etc. in the remainder of the report.