Resolving power of the microscope

Task and equipment

Information for teachers

Additional Information

In an optical microscope a first image is created through the lens which is then viewed with the aid of the eyepiece. The laws of ray optics do not impose a specific limit on the magnification brought about by the lens. However, since the light has wave properties, the theoretical magnifying power of the microscope is limited by diffraction phenomena. In transmitted-light microscope diffraction from the object and the lens must be taken into consideration. To keep the experiment simple, we have restricted it to the investigation of diffraction from the lens. In fact, both diffraction components result in a similiar resolving limit for the microscope.
The students should investigate to what extent the resolving power of the microscope is dependent a) on the wavelength of the light used and b) on the effective lens diameter. The effective lens diameter is approximately simulated by the width of the adjustable slit.

Suggestions for Set-up and Performance

For this experiment requiring several sets of measurements we recommend that students split up into groups. Each group can then carry out the measurements for one particular colour or with one particular grating.

Remark

It depends largely on the subjective perception of the observer as to whether the grating structure is judged distinguishable or not. Therefore, a fairly wide scattering of the students' results is to be expected.
Diffraction from the lens of a microscope occurs because of the circular mounting around it. In the experiment, however, a slit diaphragm is used as diffraction object, and so the results will be different to those gained from a hole diaphragm. Hence, we shall expect the results found here to deviate from those in the literature.
If the mirror box (order no. 09832-00) is available, it should be used instead of the plate mount to hold the observation lens.

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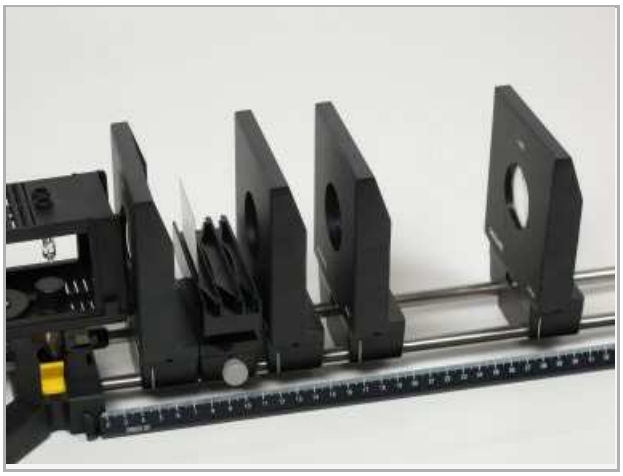
Task

What are the determing factors for the resolving power of a microscope?

1. Investigate to what extent the resolving power of a microscope depends upon the wavelength of the light used and the diameter of the lens aperture.
2. Find out how resolving power, wavelength of the light used and numerical aperture of the microscope are related.



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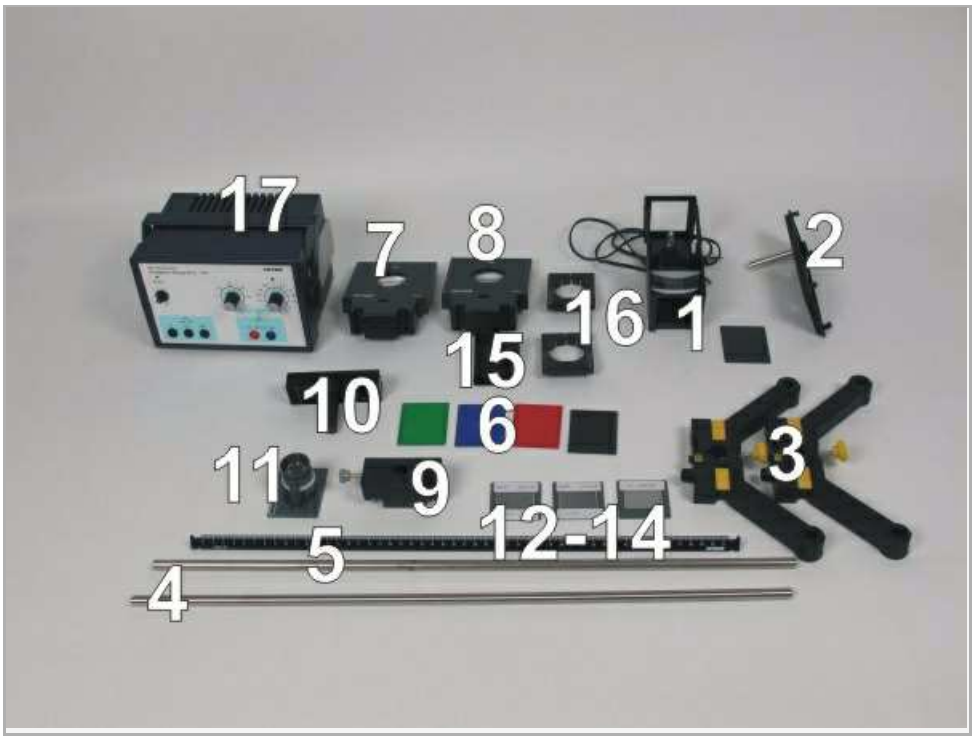
Equipment



Position No.	Material	Order No.	Quantity
1	Light box, halogen 12V/20 W	09801-00	1
2	Bottom with stem for light box	09802-10	1
3	Support base, variable	02001-00	1
4	Support rod, stainless steel, l = 600 mm, d = 10 mm	02037-00	2
5	Meter scale for optical bench	09800-00	1
6	Colour filter set, additive (red, blue, green)	09807-00	1
7	Lens on slide mount, f=+50mm	09820-01	1
8	Lens on slide mount, f=+100mm	09820-02	1
9	Slide mount for optical bench	09822-00	1
10	Plate mount f.3 objects	09830-00	1
11	Measuring magnifier	09831-00	1
12	Diffraction grating, 4 lines/mm	08532-00	1
13	Diffraction grating, 8 lines/mm	08534-00	1
14	Diffraction grating,10 lines/mm	08540-00	1
15	Slit, adjustable.up to 1 mm	11604-07	1
16	Diaphragm holder, attachable	11604-09	2
17	PHYWE power supply DC: 0...12 V, 2 A / AC: 6 V, 12 V, 5 A	13506-93	1

Set-up and procedure

- Set up the optic bench with the two support rods and the support base and place the scale in position (Fig. 1 and Fig. 2).

Fig. 1

Fig. 2

- Assemble the light box according to Figures 3 and 4 and clamp it into the left part of the support base with the lens end pointing away from the optic bench (Fig. 5). Insert a light-tight diaphragm into the well in front of the lens (Fig. 6).

Fig. 3

Fig. 4

Fig. 5

Fig. 6

- Position the lens $f = +50$ mm directly next to the light on the optic bench; attach the diaphragm holder and insert the

grating with 4 lines/mm into the holder (Fig. 7).

Fig. 7

- Place the lens with f = +100 mm at approx. 22 cm (Fig. 8).

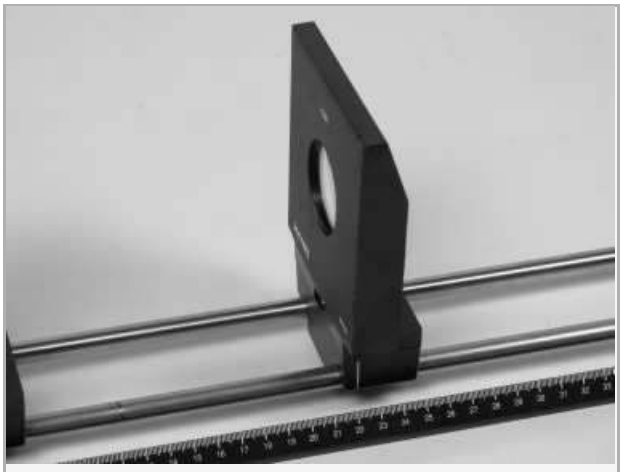


Fig. 8

- Attach the observation lens to the right-hand slot of the plate mount and set up this assembly with the slide mount at the right-hand end of the optic bench (Fig. 9).

Fig. 9

- Connect the light to the power supply (12 V~) and switch on the power supply (Fig. 10).

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Fig. 10

- Move the lens with f = +100 mm to focus the grating clearly in the observation plane.
- Insert the red filter into the light well (Fig. 11) and attach the closed adjustable slit with a diaphragm holder to the lens with f = +100 mm (Fig. 12).

Fig. 11

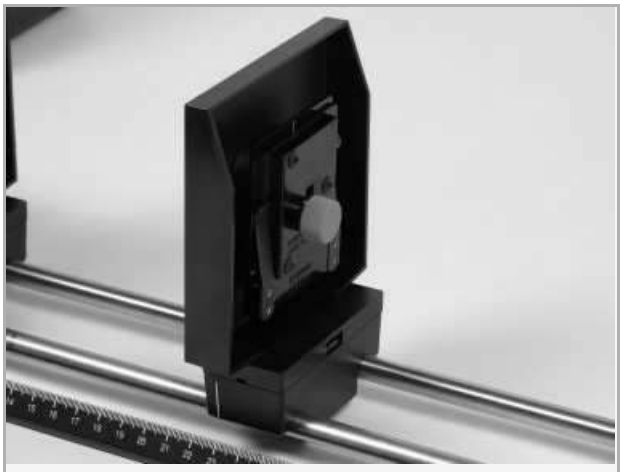

Fig. 12

- Measure and note the distance s between grating and adjustable slit.
- Increase the slit width until the line structure of the grating (object) is just visible.
- Set up a lens with f = +50 mm without grating at the position 30 cm (Fig. 13) and slide it to focus the slit diaphragm sharply in the observation plane (Fig. 14).

Fig. 13

Fig. 14

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- Measure the width d of the slit image and enter it in table 1 in the report.
- Measure the image distance *b* (distance lens - observation plane) and object distance g (distance slit - lens). Note the results in the report
- Restore the original setup and determine d for all other combinations of filters and gratings (see table 1 in the report). Enter the values in table 1 in the report.
- Switch off the power supply.

Report: Resolving power of the microscope

Result – Observations

Enter the measured values:

s = ... cm

g = ... cm

b = ... cm

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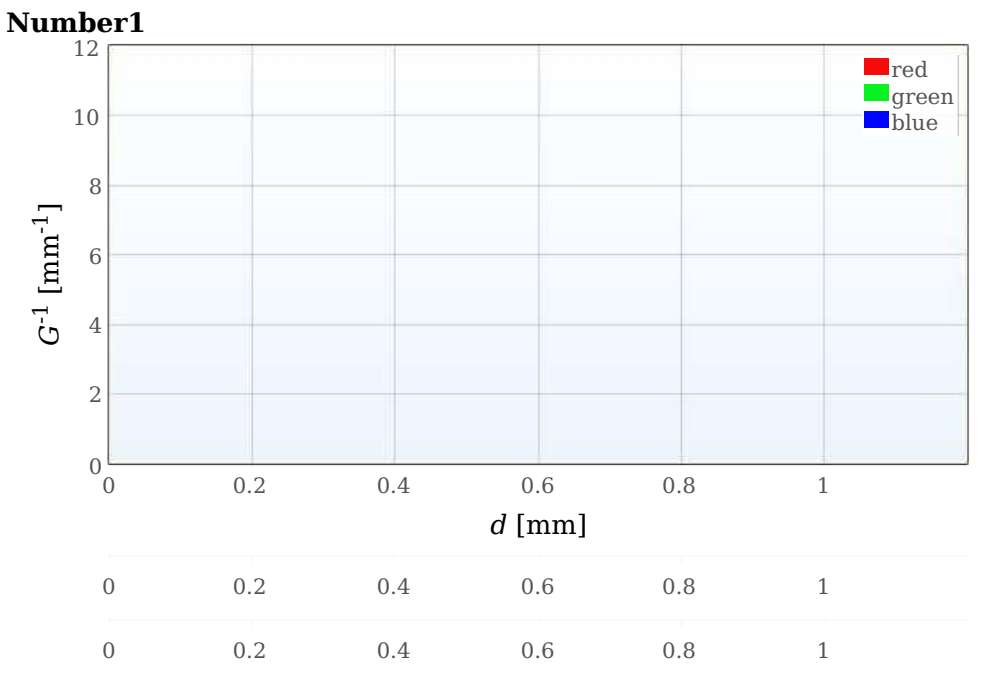
Result – Table 1

Complete the table.

The resolving limit of a microscope is the smallest distance between two object points at which they can just be perceived separately. In the experiment, this corresponds to the grating constant G (distance between two adjacent lines) of the grating used. We refer to the reciprocal value G^{-1} of the resolving limit (number of lines per mm) as the resolving power.

A graph is drawn of the resolving power G^{-1} as a function of the effective lens aperture d for the three coloured filters used (chart below).

grating	red filter		grating	green filter		grating	blue filter	
lines / mm	d' in mm	d in mm	lines / mm	d' in mm	d in mm	lines / mm	d' in mm	d in mm
4	1 ±0.1	1 ±0.1	4	1 ±0.1	1 ±0.1	4	1 ±0.1	1 ±0.1
8	1 ±0.1	1 ±0.1	8	1 ±0.1	1 ±0.1	8	1 ±0.1	1 ±0.1
10	1 ±0.1	1 ±0.1	10	1 ±0.1	1 ±0.1	10	1 ±0.1	1 ±0.1



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Evaluation – Question 1

What conclusion can we draw from the chart under "result - table 1" on the correlations between the resolving power G^{-1}, the critical size of the lens aperture d and the wavelength of the light used?

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Evaluation – Table 2

The numerical aperture *A* as lens indicator is half of the angle at which the critical lens aperture is visible from the object.

$$A = n \text{ x } \sin(\alpha/2) \approx d \text{ / } 2s \text{ (Fig. 15).}$$

There is air located in the gap between object and lens, and therefore in the experiment performed *n* has the value 1. Calculate the aperture *A* for all cases investigated and enter the values in the table.

Grating	red filter		green filter		blue filter	
G^{-1} in mm^{-1}	*A*	*G* x *A* in nm	*A*	*G* x *A* in nm	*A*	*G* x *A* in nm
4	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0
8	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0
10	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0	1 ±0

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Evaluation – Question 2

What correlation between resolving power and numerical aperture is evident from table 2?

Evaluation – Question 3

Compare the products G x A with the wavelength of the respective light used.

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Evaluation – Question 4

Explain the limited resolving power of a microscope on the basis of diffraction of light from a lens aperture. Use Fig. 16 to help you.

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