

MEASUREMENTS OF DIMENSIONS OF SMALL OBJECTS BY MICROSCOPE

Name:

Group:

Date:

1. Goal of the experiment:

2. Magnification and resolving power of the microscope:

Magnification of the objective $m_o =$

Magnification of the eyepiece $m_e =$

Total magnification of the microscope $m_t =$

Wavelength of light used for observation: $\lambda =$

Numerical aperture of the objective: $A =$

Minimum distance between two points of the object that can be seen as separate: $d =$

Resolving power of the microscope $RP =$

Check if the total magnification m_t is within the practical magnification limit ($500 \cdot A < m_t < 1000 \cdot A$):

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3. Calibration procedure - determination of the calibration coefficient k :

The smallest graduation of the standard scale of stage micrometer: $b = (10.00 \pm 0.05) \mu\text{m}$

Number of divisions b of the reference line segment $N_1 =$

Length of the reference line segment $s \pm \Delta s =$

Maximum length of the reference line segment in pixels $l_{maks} =$

Minimum length of the reference line segment in pixels $l_{min} =$

Average length of the reference line segment in pixels $l =$

Error of the average length of the reference line segment in pixels $\Delta l =$

Calibration coefficient in standard units of length (μm) $k =$

Error of the calibration coefficient $\Delta k =$

$k \pm \Delta k =$

EXPERIMENTAL PROCEDURE

1. Turn on the computer with the software for microscope image capture and check whether the digital camera of the microscope is connected to the computer USB input.




2. Start the OptaView IS computer program (ikon).
3. Turn illuminator on in order to illuminate the specimen.
4. For observation chose the objective of the smallest magnification (10×).
5. Rotate coarse focus knobs (see Fig. 1) in a direction that stage (2) moves “away” from the objective lenses (3) as far as possible and position the stage micrometer on the stage:
 - swing movable finger on slide holder (4) outward, place the stage micrometer (cover slip facing up) on top of stage against fixed side of slide holder,
 - slowly release movable finger until it makes contact with stage micrometer side,
 - using mechanical stage X and Y controls (5) move the stage micrometer until it is centered over the condenser lens (6).

Raise the stage by rotating the coarse focus knob backwards until the tip of the lens is slightly above the stage micrometer. (Be careful not to touch the objective lens to the slide).

6. Turn the focus adjustment knobs (1) slowly until the stage micrometer comes into sharp focus and, using mechanical stage X and Y controls (5), move stage micrometer until it is centered in the field of view.
7. While looking through the eyepieces close the field diaphragm (7) located on top of light collecting lens assembly (8) to about 1/3 of the field. Focus the field diaphragm of the illuminator by lowering the condenser lens. Iris leaves should be focused.
8. Changing magnification is accomplished by rotating objective nosepiece (10) until different objective lens is moved into optical path. Rotate revolving nosepiece to position 40x and using focus adjustment knobs bring stage micrometer into sharp focus. Repeat the procedure described in point 7. Finally, rotate revolving nosepiece to position 60x (target magnification) and bring stage micrometer into sharp focus and again repeat the procedure described in point 7.
9. Optimize the image quality by adjusting the aperture diaphragm (11) (i.e. its opening). It controls the amount of light, helps to achieve high resolution of specimen and provide contrast in the image. Smaller apertures will deliver higher contrast to image. However, closing aperture too much will reduce resolution (!). Experimentation is the best method for determining the correct opening of diaphragm.
10. Take a picture of the stage micrometer image.

11. Calibration procedure:

- a) measure the length of line segment of the greatest possible length l_{max} then measure the length of line segment of the smallest possible length l_{min} , corresponding to the selected reference line segment s – see Fig. 2 (in this figure s equals $17 \times 10 \mu\text{m} = 170 \mu\text{m}$) - MENU: measurement, tool: . Calculate the average value of the length l and its error Δl :

$$\Delta l = \frac{l_{max} - l_{min}}{2}$$


- b) calculate value of the calibration coefficient k , which converts pixels into μm :

$$k = \frac{s}{l}$$

and estimate its error Δk .

12. Follow the above description (points 4 through 9) and bring the image of erythrocytes into sharp focus. Magnification must be the same as that during the calibration procedure i.e. 60×.

13. Take a picture of the image of erythrocytes and make measurement of diameters of 10 randomly chosen

erythrocytes - MENU: measurement, tool .

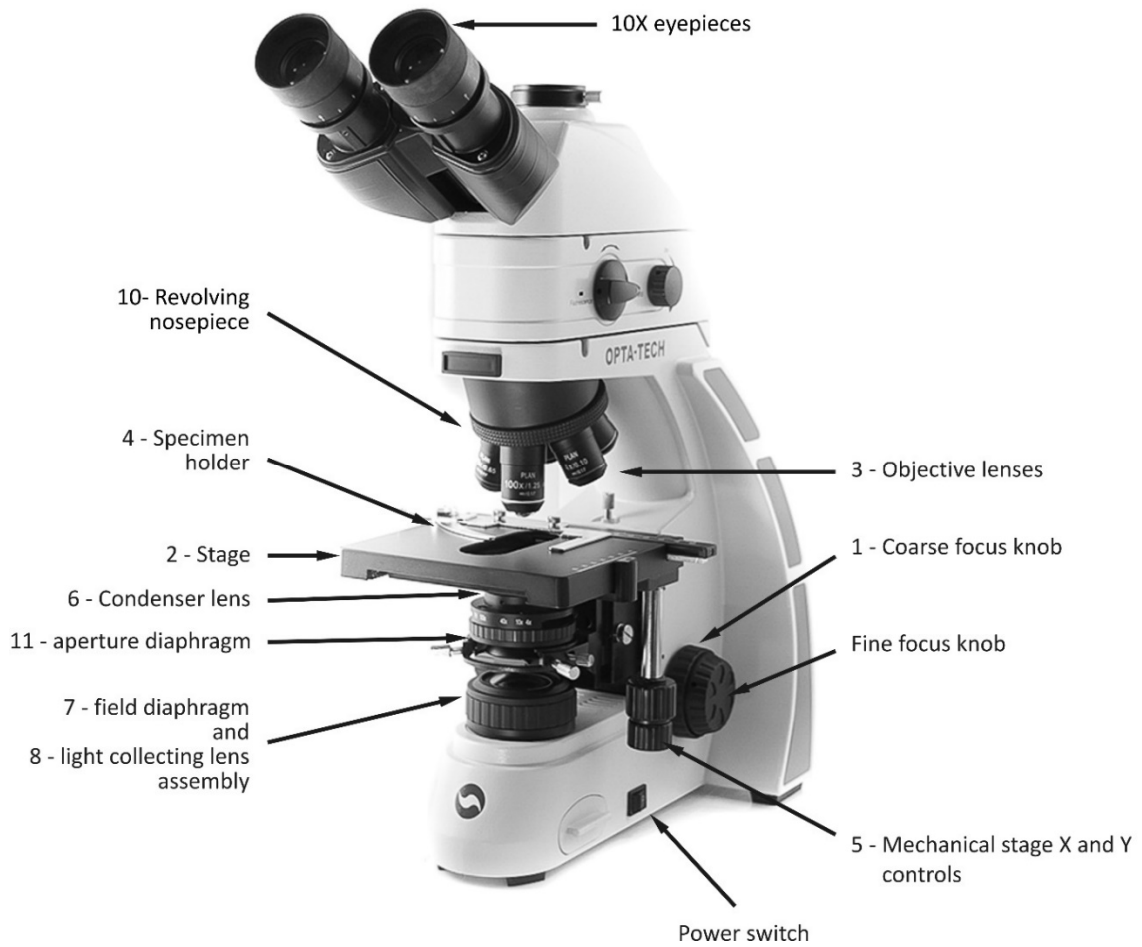


Fig. 1. Components of the microscope.

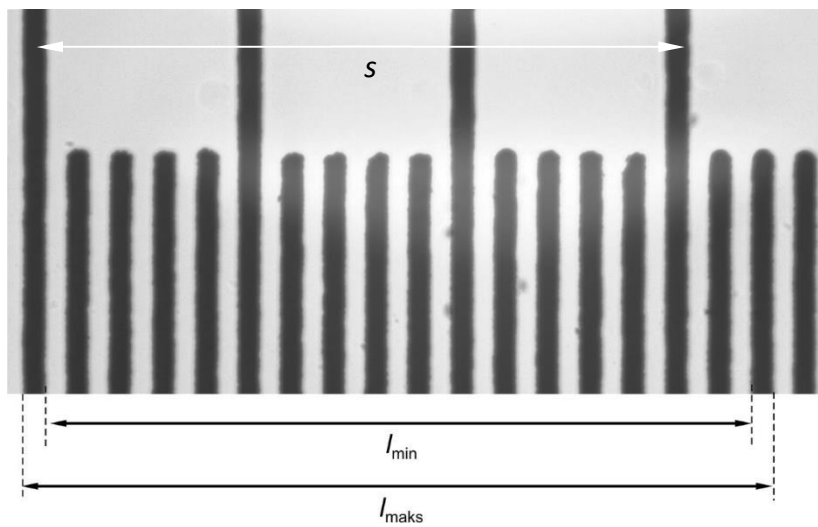


Fig. 2. Image of the stage micrometer.