For General Medicine

Topic of section: Microscopy

Topic of lab work: The measurement of small objects with a microscope

Aim: calibrate an ocular micrometer, measure the size of small objects with a microscope.

Theory:

1. Write a formula of thin lens. How is a thin lens magnification determined?
2. Plot a ray tracing in the optical microscope.
3. Write a formula for optical microscope magnification.
4. Explain a microscope resolution limit determination. Write Abbes' formula. Give a numerical value for optical microscope resolution limit.
5. What is the difference between magnification and resolution?
6. What is the maximum magnification and resolution normally achievable with a compound light microscope?
7. How does the wavelength of light affect the resolution of a microscope?
8. What is the purpose of calibrating an ocular micrometer with a stage micrometer?

Practical part:

All measurements of length are based on a comparison of the object under scrutiny with another of known dimensions, or with a standardized, calibrated scale. This basic principle is applicable to the measurement of specimens observed in the microscope.

The purpose of this lab work is to calibrate a scale (an ocular micrometer) in the eyepiece of the microscope. It can be used to measure the dimensions of different types of microscopic objects. The ocular micrometer scale has no inherent units, and

because different objectives produce images with different degrees of magnification, the meaning of its intervals varies from one objective to another.

Because the ocular micrometer has no inherent units, it is necessary to calibrate it using a stage micrometer (the camera of Goryaev). A stage micrometer is a special microscope slide with a ruler etched on its surface, which has units of millimeters (mm) and micrometers (μm). To calibrate ocular micrometer, you will line up the stage micrometer with the ocular micrometer and count the number of units or divisions on the ocular micrometer that corresponds to a particular distance in millimeters or micrometers on the stage micrometer (Figure 1). The number of ocular units per millimeter or micrometer will change as the magnification changes.

Setup procedure:

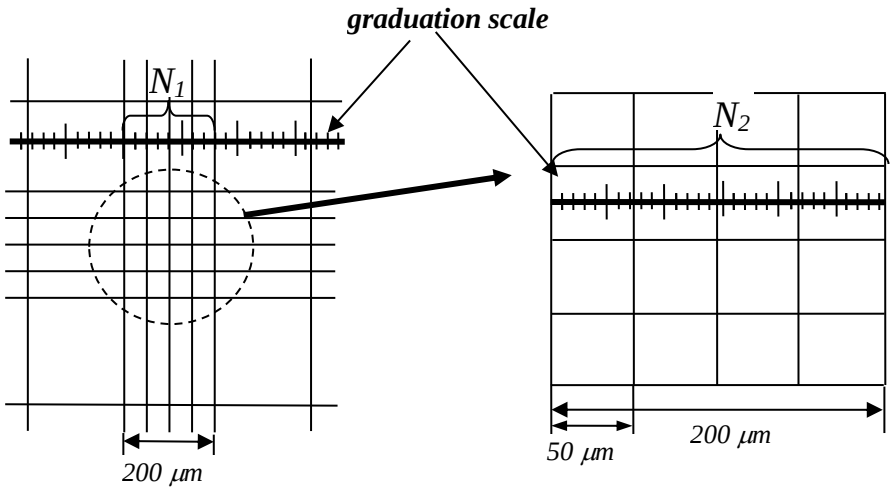
1. **Calibration of the eyepiece graduation scale**
    1. Set in the microscope objective with an 8-fold magnification.
    2. Rotate the 8-fold objective lens of one of the microscopes fitted with an ocular micrometer into position. Obtain a stage micrometer and position it on the stage. Be sure that the scale is on the top side of the slide. Adjust the illumination and bring the image of the stage micrometer into focus.
    3. Determine the value of division of the graduation scale for the 8-fold microscope objective by formula:

$$S_8 = \frac{200\mu m}{N_1}$$

where N is the number of the graduation scale divisions corresponding to the four smallest mesh strips (4x50μm) of the stage micrometer.

    4. Determine the value of division of the graduation scale for the 40-fold microscope objective by formula:

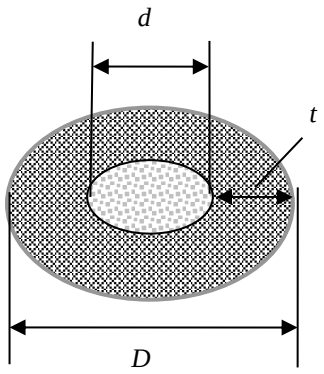
$$S_{40} = \frac{200\mu m}{N_2}$$



2. Determination of object dimensions

1. Use 8-fold objective. Determine the dimensions of the hair cross-section.
2. Determine the external diameter ***D*** of hair according formula:

$$D_{hair} = NS_8$$



3. Determine the internal diameter ***d*** of hear similarly.
4. Calculate the epithelium thickness ***t*** as difference between two previous values.
5. Use 40-fold objective. Determine an erythrocyte diameter:

$$d_{erythr} = NS_{40}$$

6. Write conclusion.

Solve problems:

1. In the microscope with 500-fold magnification the focal length of the eyepiece is equal to 4 cm and the length of the optical tube is equal to 20 cm. Find the focal length of the objective.

   Answer: 0.25 cm
2. The minimum resolution limit in the optical microscope is equal to 0.3 μm for wavelength of 450 nm. Find the minimum resolution limit for the wavelength of 600 nm.

Answer: 0.4 μm

3. The resolution limit in the optical microscope without immersion is 0.45 μm. Find the resolution limit when the immersion fluid with a refractive index of 1.5 is used.

   Answer: 0.3 μm

Literature

1. Optical, electron and atomic force microscopies: учеб.-метод. Пособие / Л.В. Кухаренко, О.В. Недзьведь. – Минск: БГМУ. 2014. – 31 с.