7.3. MEASUREMENTS WITH MICROSCOPES

Purpose of experiment

- To measure the magnification of a microscope;
- To measure linear dimensions of objects and parameters of biological objects

Theoretical topics

- Structure of a microscope and ray traces in a microscope
- Microscope magnification
- Microscope resolution and magnification abilities
- Applications of microscopes
- Other types of microscopes

Equipment and material

Microscope (eyepiece with micro-grid), micro-ruler, histological samples, MOTIC camera, computer, software for image analysis

Methodology

The experiment is conducted with a microscope generally as shown in Fig. 7.3.1. and the optical setup in Fig. 7.3.2. One rotation of the knob (2) corresponds to a 0.5 mm shift of the tube. If the coarse adjustment (4) and microscope focusing (2) knobs are rotated clockwise when the eyepiece faces the observer, the microscope tube moves downward, and if counter clockwise – rises upward. An eyepiece with a micro-grid is mounted on the tube of the microscope used in this experiment. The micro-grid single interval is known: c = 0.1 mm.

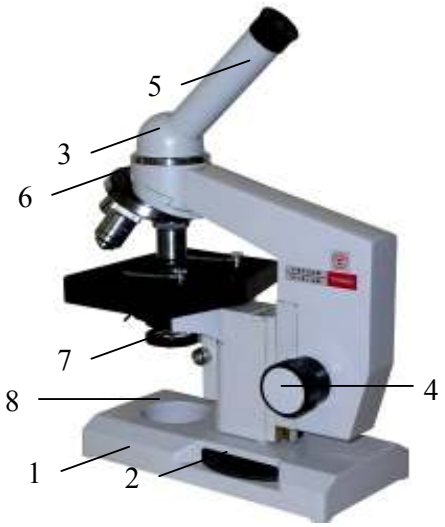

Fig 7.3.1. Microscope: 1 – microscope base , 2 – disc shape micrometer focusing knob, 3 – tube holder, 4 – coarse adjustment knob, 5 – tube, 6 – revolving nosepiece, 7 – two lenses condenser, 8 – light source.

The microscope's optical set-up consists of two parts (Fig. 7.3.2.): 1) lighting, consisting of the mirror (1) and condenser (2) with aperture diaphragm (3) and 2) observation, consisting of the objective (4), additional lens (5), the prism system (6), prisms (7) and the eyepiece (8). The sample is placed on the stage (9) below the objective.

A light beam from the natural or artificial light source falls on the mirror (1), which directs the beam to the diaphragm (3). The light beam then goes through the condenser (2), the research sample and enters the objective lens (4). The prism (7) tilts the beam at a 45° angle

from the vertical for more comfortable observation of the sample. The central prism directs light to the eyepiece (8).

Dotted lines represent rays which form the central point of the research object image, and the solid lines – rays which are transmitted through the microscope visual field edges.

Evaluation of microscope magnification and dimensions of the object

The magnification of the selected objective (it is advisable to start using the lowest magnification objective and later use the revolving nosepiece to select the correct one) is measured with a micro-ruler – a transparent glass plate with equidistant lines (Fig 7.3.3.). A micro-ruler interval (distance between the nearest two lines) is a = 0.1 mm. The plate is placed on the microscope table. A sharp image of the micro-grid and crossed lines is obtained by rotating the eyepiece counter clockwise or clockwise. The smallest possible distance between the objective and the sample is then obtained by carefully turning the coarse adjustment knobs (**not touching the micro-ruler or sample!**). A sharp micro-ruler image is focused by slowly rotating the microscope knob in the opposite direction and observing the object through the eyepiece. It is advisable to start by using the lowest magnification objective and later replacing it with the right objective by using the revolving nosepiece.

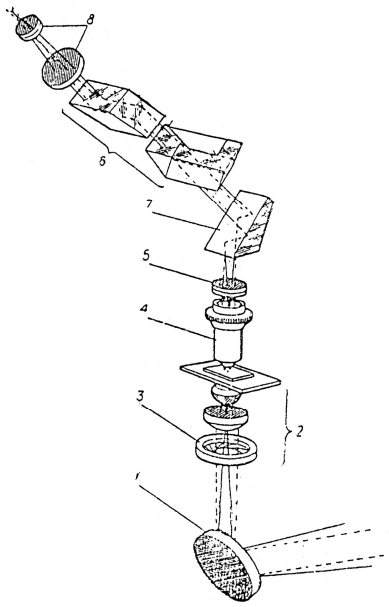

Fig. 7.3.2. Microscope setup: 1 – mirror, 2 – condenser, 3 – aperture diafragm,4 – objective, 5 – additional lens, 6 – prism system, 7 – prism, 8 – eyepiece, 9 – stage.

Fig. 7.3.3. Micro-ruler image

Procedures

1. **Microscope with an eyepiece with a micro-grid**

This is a microscope with an eyepiece equipped with a micro-grid. One interval of the micro-grid is c = 0.1 mm.

1. Properly orientate the micro-grid and obtain a focused image of the micro-ruler by rotating the coarse and fine adjustment knobs. Looking through the eyepiece, count how many intervals m of the micro-grid correspond to n micro-ruler intervals (n is selected by the experimenter, depending on the objective magnification and the number of intervals which can be seen through the eyepiece).

2. Calculate the linear magnification of the microscope objective:

$$N_o = \frac{mc}{na}, \qquad (7.3.1)$$

where *n* micro-ruler intervals and a = 0.1 mm or *na* is size of the reference object (black spot) on the micro-ruler (the size is written on the micro-ruler).

3. Place and properly orient the sample on the microscope stage and the form a clear image. Count the number *m* of the micro-grid intervals that corresponds with the object.
4. Calculate the size of the object *L*

$$L = \frac{cm}{N_o}. \qquad (7.3.2)$$

2. Microscope with MOTIC camera

a

b

c

Fig. 7.3.4. MOTIC camera

Microscope with MOTIC camera

Microscope with MOTIC camera and computer

1. Switch on the computer and the light source of the microscope with a MOTIC camera (c in Fig. 7.3.4.).
2. Start the program *Motic Images Plus* and from the menu choose *File> Capture Window*. A live view from the microscope objective can then be seen in the window.
   Calibration (steps 3-6) should be carried out with the assistance.
3. Calibration is performed with an object of known size. A micro-ruler with known intervals or round spots of known diameter can be used as objects of known size. An object is placed on the microscope stage and a sharp image is obtained. The image is saved selecting *Capture> Still Image*.
4. From the menu select *Measure> Calibration Wizard*. If you perform the calibration with a round spot, choose *Calibrate with calibration circle,* if with the micro-ruler - *Calibrate with Scale Cross* and load your image by clicking the *Load Image* icon.

5. When calibrating using a round spot, enter the diameter of the spot in the field and click *Calibration*. When performing calibration, the centre of the circle must coincide with the scale intersection point in the image, and the edges of the circle (or ellipse) must coincide with the selected number of scale intervals (horizontal and vertical). In the appropriate fields (*Width* and *Height*) add the distance from the intersection point to the circle (ellipse) points, crossing the scale and click *Calibration*. Select the name of the calibration and save it.
6. Run *File> Capture Window*, and obtain sharp images of the samples (at least 3 different samples of blood, hair). Images are saved by selecting *Capture> Still Image*.
7. Select the image for analysis and from the bottom menu bar select *Measure.* From the drop down menu select your saved calibrations. In the top menu, select *Measure> Line* and measure the size of the selected object.
8. The results are compared with the data presented in tables.