

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/337635347>

Practical 6. The measuring of microscopic objects using microphotograph scale bar, field of view diameter and ocular screw micrometer

Chapter · December 2019

CITATIONS

0

READS

495

3 authors:



Boris M. Sharga

Uzhhorod National University

60 PUBLICATIONS 85 CITATIONS

[SEE PROFILE](#)



Diana B. Pylypiv

15 PUBLICATIONS 0 CITATIONS

[SEE PROFILE](#)



Volodymyr Feketa

Uzhhorod National University

58 PUBLICATIONS 4 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Bacteriorhodopsin production [View project](#)



Pesticides determination [View project](#)

Uzhhorod National University
Medical Faculty №2
Department of Fundamental Medical Disciplines

MEDICAL BIOLOGY PRACTICALS. CYTOLOGY.

Practical 6. The measuring of microscopic objects using microphotograph scale bar, field of view diameter and ocular screw micrometer.

By Boris M. Sharga, Diana B. Pylypiv, Volodymir P. Feketa

Objectives of this practical lesson are to learn some methods of measuring of microscopic objects and to gain an experience in procedure for calibration of the ocular screw micrometer and estimation of linear magnification of objective for accurate sizing of cells.

Theoretical background. In practice, the size of objects in microscopy is important for diagnostics and can be measured in several ways. In work with optical microscope, this can be carried out by applying the methods based on using of the field of view diameter (FOVD) size as a scale; the hemocytometer squares side size as a scale bar; the ocular micrometer (OM) calibrated scale; the ocular insert for full stereological measurements of microscopic objects [3]; the ocular screw micrometer (OSM) [1, 2]; the electronic micrometer eyepiece and the image analysis system.

Sophisticated microscopes, such as scanning electron microscope (SEM), transmission electron microscope (TEM), some computerized optical microscopes, etc., generate scale bars allowing estimation of observed structures sizes (Fig. 1).

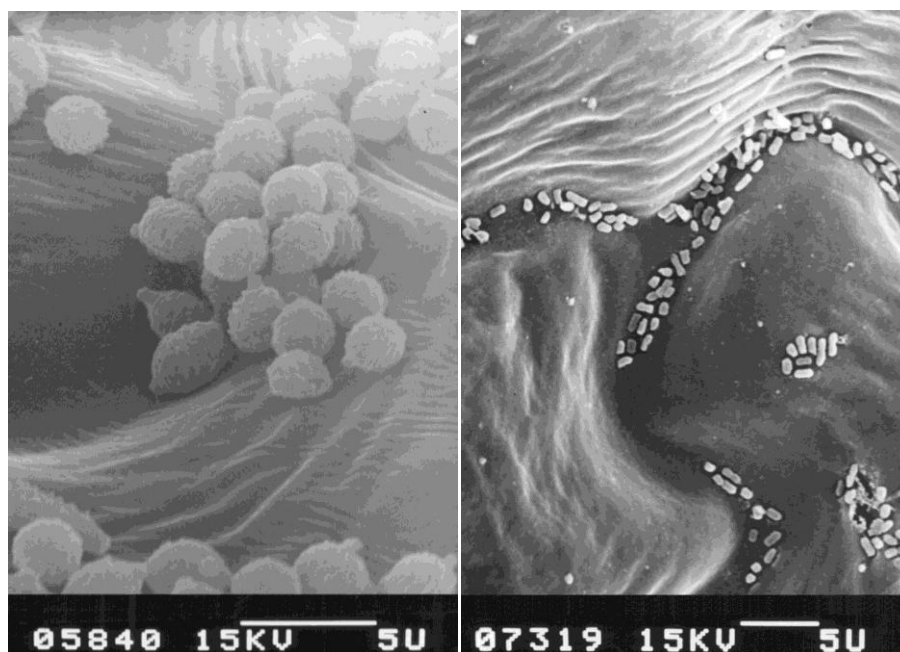


Fig. 1. *Streptomyces* sp. spores (left) and *Bacillus subtilis* cells (right) in SEM Hitachi S430. By Boris M. Sharga.

The OSM has stationary scale with marks from 0 to 8 mm and moveable cross and index in shape of two parallel lines starting from upper field of view margin. They are visible in focal plane of the OSM ocular (Fig. 2). The OSM consists of box 2, base muff 5 for joining with tube of microscope, ocular 1 with double optic mechanism (Fig. 3), fixed slide 8, counting attachment, consisting of micrometer screw 9 and restrictive nut 10, counting knob 4, slider 6 with moveable slide 7 (Fig. 4). The stationary scale is printed onto transparent stationary slide 8, the moveable cross and index made onto slide 7. The rotation of the screw 9 by knob 4 of the micrometer causes the cross and index to move relatively to the stationary scale (Fig. 4). The screw 9 step is equal 1 mm, so one full rotation of the knob 4 results in movement of the index and cross on one mark (1 mm). Thus, stationary

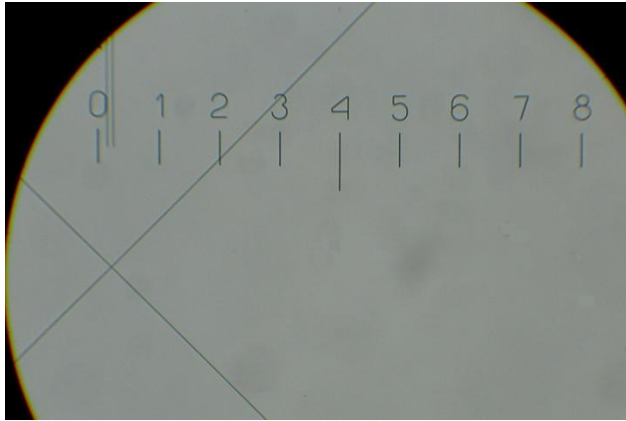


Fig. 2. OSM stationary scale, moveable index and cross. By Diana B. Pylypiv.

scale is serving for counting of full rotations of the knob 4, i. e., millimeters. Because the knob has 100 marks, its rotation on 1 mark moves the cross and index within the field of view on 0.01 mm (10 μ m). Thus, the scale of the knob 4 is used to count the decimal and hundredth fraction of millimeter. The complete count of the micrometer consists of sum of count from stationary scale and from the knob 4.



Fig. 3. Parts of the OSM. By Diana B. Pylypiv.

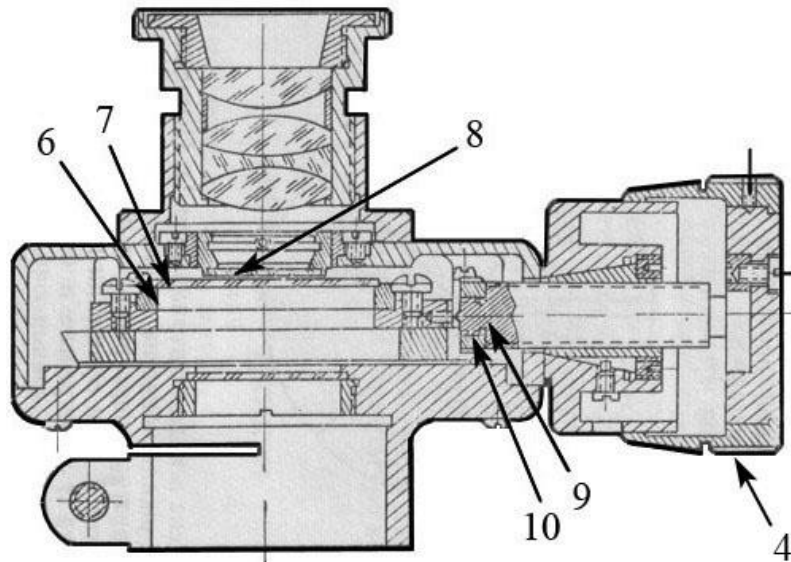


Fig. 4. Internal structure of the OSM.
Modified from [2] by Diana B. Pylypiv.

The count from stationary scale depends from the position of the index on the scale relative to “0” mark. The count onto knob is estimated by mark superimposed with index, printed onto stationary cylinder. For example, the moveable index is situated between the marks “4” and “5” of stationary scale and reading from the knob scale against stationary index is “55”. So we have 4 mm from the stationary scale and $0.01 \times 55 = 0.55$ mm from the knob of the screw. The complete reading is: $4 + 0.55 = 4.55$ mm. If index is situated between the marks “2” and “3” of stationary scale and reading from the knob scale against stationary index is “4”, the complete reading is $2 + 0.04 = 2.04$ mm. If index has position pictured on Fig. 2 and reading from the knob scale against stationary index is “20”, so the total reading is “0.20”.

Experiment 1. Use of microphotographs scale bar for measurements

Materials and Equipment

Photoes from scanning electron microscope with scale bars, transparent ruler.

Procedure

1. Apply the scale bar ($5U = 5 \mu\text{m}$) in Fig.1 to measure the average size of the *Streptomyces* sp. spores (Fig.1, left) and *Bacillus subtilis* cells (Fig.1, right). Use the transparent ruler to measure scale bar and sizes of the microbes on photographs in mm. Then use the formula to calculate x , the large diameter of spores and length bacterial cells: $x = \frac{l \times 5}{L}$, where L is a length of scale bar on photograph in mm, l is an average length of microbe on photograph in mm and 5 is for scale bar $5U = 5 \mu\text{m}$.
2. Use the scale bar to calculate the squares of these and other photos and number of bacterial sells or number of other microscopic objects per mm^2 of the tissue at home alone to present the data next time in class.

Results

The x is approximately equal $2.8\ \mu\text{m}$ for actinomycete spores (Fig.1, left) and $1.3\ \mu\text{m}$ for the rod-shaped bacterial cells on right part of the Fig.1.

Experiment 2. The estimation of FOVD size and its use as a scale

Materials and Equipment

Transparent ruler, compound light microscope, glass slide preparations of microscopic objects.

Procedure

1. Place transparent ruler onto stage of microscope under objective $4\times$ and ocular $10\times$. The magnification is $4 \times 10 = 40\times$.
2. Count millimeters number fitting into FOVD. From Fig. 5, you may estimate the FOVD as 5 mm approximately ($\approx 5000\ \mu\text{m}$).
3. It is not necessary to use the ruler each time when you need to estimate the FOVD for higher magnifications. For example, if we have changed ocular on $15\times$ with same objective $4\times$ we have now higher magnification, $60\times$. The FOVD for this magnification can be calculated from the equation: $5000 \times 40 = X \times 60$, thus $X = (5000 \times 40) : 60 \approx 3333\ \mu\text{m}$. If we use now objective $10\times$ and ocular $10\times$, so we have magnification $100\times$ and equation: $5000 \times 40 = X \times 100$, thus $X = (5000 \times 40) : 100 \approx 2000\ \mu\text{m}$.
4. Now you may roughly measure length of large objects using FOVD as a scale bar.



Fig. 5. FOVD measurements using transparent ruler $\approx 5000\ \mu\text{m}$, $40\times$.

By Diana B. Pylypiv.

For example, it is possible to measure the size of fungal hyphae stretching along diameter of field of view or estimate approximate dimensions of human parasite microscopic stage, etc. in supplied preparations.

Results

Example: The length of *Botrytis* fungus germ tube ranges from, approximately, 250 to $1000\ \mu\text{m}$ in

provided preparations.

Experiment 3. The objective linear magnification estimation using OSM and SM

Materials and Equipment

OSM, stage micrometer, compound light microscope.

Procedure

1. Remove the eyepiece from the microscope tube and join the muff of the OSM with the tube.

2. By rotating the ocular of OSM find the best view of the cross, index and stationary scale.
3. Select the distance onto scale not larger than the 2/3 of the diameter of field of view. It must be down in its central part, where all lines more clearly visible than onto periphery.
4. Move the index to the mark “0” of the stationary scale.
5. Place the stage micrometer (SM) (Fig. 6) onto the stage of microscope and looking through ocular of OSM find the cycle with scale in the centre of it.

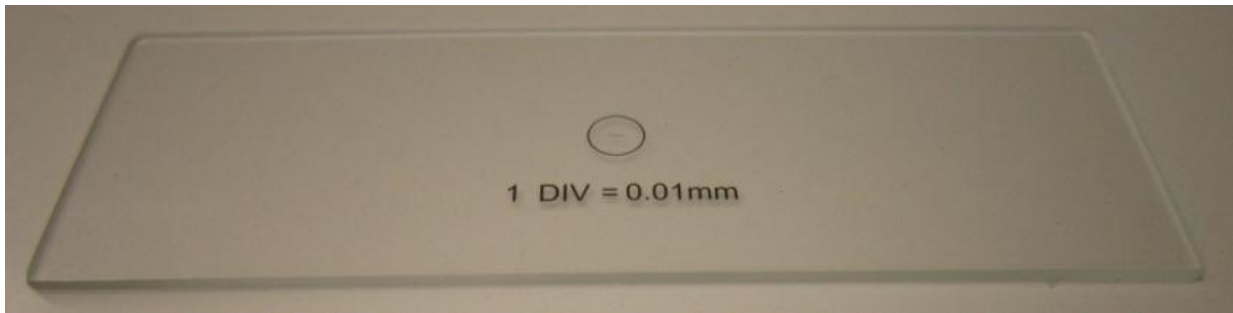


Fig. 6. Stage micrometer with scale mark value 0.01 mm.

By Diana B. Pylypiv

6. By moving of the stage position the first mark of the SM onto distance of its 2-3 marks from the cross center. The marks of the SM must be in parallel with 2 lines of OSM index (Fig. 7).
7. By observing into ocular and rotating the knob 4, superimpose the cross center with first mark of the SM. Note the first count, **A** from the OSM stationary scale and knob readings.

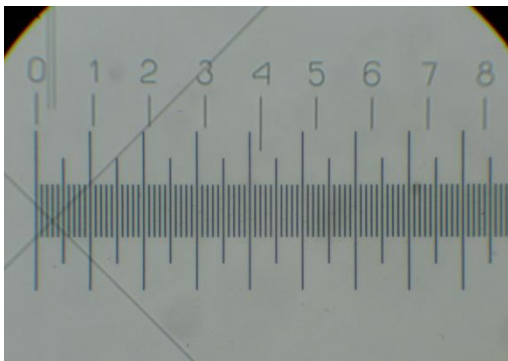


Fig. 7. Matching the scales of OM and OSM. *By Diana B. Pylypiv.*

Then, by rotating the knob 4 superimpose the cross center with another mark of the SM. This mark is situated approximately on the same distance from the margin, but onto opposite side of the field of view. Note the second count, **B** from the OSM scale and knob readings. (Alternatively, two marks can be selected on SM by moving the cross in opposite direction, i.e., from “8” to “0” of OSM stationary scale). Estimate the difference between these two counts by

subtracting lower number from the higher [**A - B**].

8. Count **z**, the number of the SM marks corresponding to distance between **A** and **B**.
9. Calculate β , the linear magnification of the objective by using the formula:

$$\beta = \frac{[A-B]}{z \times a}, \text{ where:}$$

[**A - B**] – the difference between two OSM counts;

z - the number of the marks of SM corresponding to distance between A and B ;
 a – the value of 1 mark of SM, 0.01 mm.

Results

Example: First count on OSM is $A = 6.55$ mm and second count on OSM is $B = 4.05$ mm. The number of marks of object micrometer, z , corresponding to distance between A and B is 25. The linear magnification of the objective can be calculated:

$$\beta = \frac{[A-B]}{z \times a} = \frac{6.55-4.05}{25 \times 0.01} = 10.$$

Thus, the magnification of the objective is $10\times$.

Experiment 4. The estimation of the microscopic object size using OSM

Materials and equipment. OSM, compound light microscope, glass slide preparations of microscopic objects.

Procedure

1. Substitute the SM by the glass slide with preparation of squamous cells from throat lining, onion epidermis or fungal mycelium.
2. Focus the microscope to see well the object you want to measure.
3. By observing into ocular and rotating the knob 4 clockwise, superimpose the cross center with one end of the object. Note the first count, A from the OSM stationary scale and knob readings. Then, by rotating the knob 4 superimpose the cross center with another end of the object. Note the second count, B from the OSM scale and knob readings. Estimate the difference between these two counts $[A-B]$.
4. Estimate the size of object by the equation:

$$l = \frac{[A-B]}{\beta}, \text{ where:}$$

$[A - B]$ – the difference between two OSM counts and β is the linear magnification of the objective.

Results

Example: The counts from scale of OSM micrometer when cross center was sequentially superimposed with one and another ends of the object (human buccal cell) is 1.25 and 2.37 mm. So $[A-B]$ equals 1.12. The magnification of the objective is $10\times$ from previous calculations (Experiment 3). Thus, the size of the cell can be calculated as:

$$l = \frac{[A-B]}{\beta} = \frac{1.12}{10} = 0.112 \text{ mm} = 112 \mu\text{m}.$$

Some time it is more suitable to measure the size of the object by another way. Estimate ε , the value of one mark of the knob scale into plane of OSM ocular view by equation:

$$\varepsilon = \frac{0.01}{\beta}.$$

The 0.01 is a mark value of the knob 4. The magnification of microscope β in our example is equal 10, thus

$$\varepsilon = \frac{0.01}{10} = 0.001 \text{ mm.}$$

The size of the object is calculated by the equation:

$$l = \varepsilon [A - B].$$

Here $[A - B]$ is a difference between two OSM readings in knob 4 units.

Thus, l , the buccal cell size can be calculated from the *Example* of Experiment 4 using OSM readings as:

$$l = \varepsilon [A - B] = 0.001 \times [237 - 125] = 0.001 \times 112 = 0,112 \text{ mm} = 112 \text{ }\mu\text{m}.$$

Literature:

1. Waelsch J.H. Measurement of microscopic objects with the aid of an ocular screw micrometer with the reticle branch// Zeitschrift für medizinische Labortechnik.- 1967.- Vol. 8, №3.- P. 178-180. [Article in German].
2. Микрометр окулярный винтовой МОВ-1-15. Техническое описание и инструкция по эксплуатации.- 1978, ЛОМО, Ленинград. -12 с.
3. Стефанов С.Б. Окулярная вставка для полных стереологических измерений микроскопических объектов// Цитология.- 1974.- т. 16, №1.- P.1439-1441.