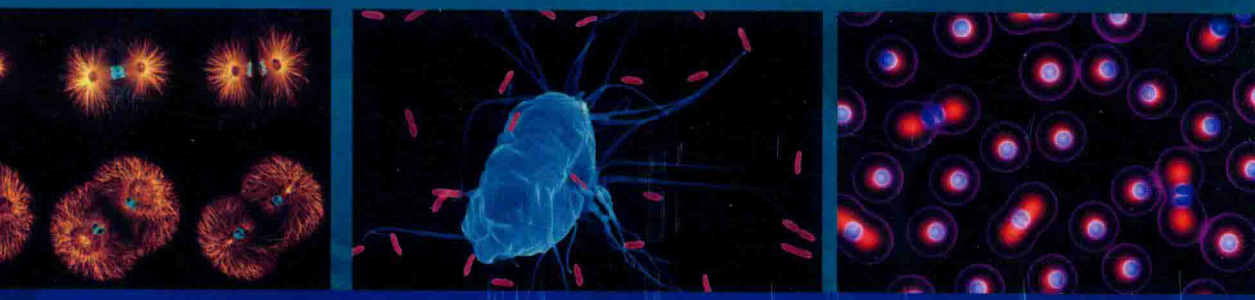
高等院校“十三五”规划/创新实验教材系列：医药类

Experimental Guidance for Cell Biology

细胞生物学实验指导



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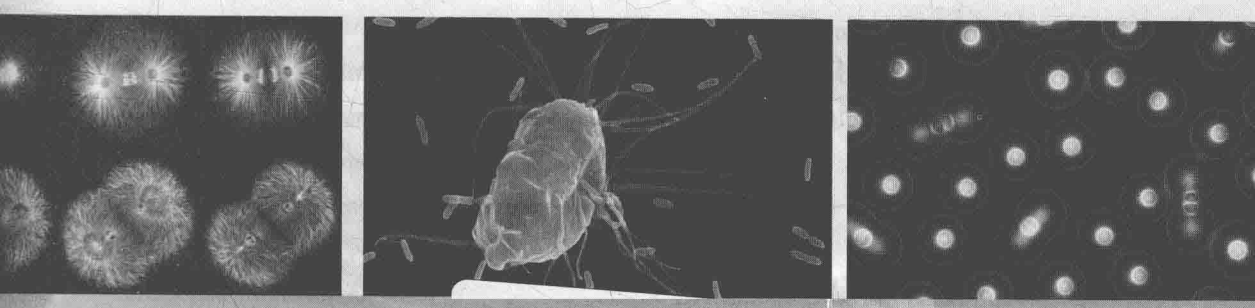

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Preface

I was very pleased to get financial support from Facility Division of Sun Yat-Sen University to compile and publish this Experimental Guidance for Cell Biology. Zhongshan Medical School of Sun Yat-Sen University has enrolled international students for several years, and we always lack a published English version of traditional experimental guidance for Cell Biology.

We recruited eight traditional experiments in this experiment manual which are suitable for all Chinese medical students in learning the experimentation of Cell Biology. That means, this experiment manual is not only appropriate for MBBS (Medical Bachelor and Bachelor of Surgery) students of our medical school, but also suited for Medical students of Eight-year MD program or Five-year program.

Associate Professor Zhong Xiaomin and Ke Qiong have participated in compiling this experiment manual, and Instructor Luo Ying and Ren Sijin from School of Foreign Langnages have helped in revising. I want to express my heartfelt gratitude to these ladies.

I am also particularly grateful to Facility Division of Sun Yat-Sen University for the fund to make this book a reality. Last but not least, Chief Editor Zhou Jianhua and Editor Xie Zhenjing of Sun Yat-Sen University Press strongly support the publishing of this manual; without them, the publication would be postponed.

From the editor

May 24th, 2017

Contents

Experiment 1 The Structure and Usage of the Optical Microscope

Ⅰ. Experimental Purposes

(1) Be familiar with the structure of optical microscope.

(2) Learn to use low power lens, high power lens and oil lens.

Ⅱ. Experimental Principle

Optical microscope contains mechanical parts and optical parts. The optical parts are important, containing the objective, the ocular, the condenser and the light source, etc. The objectives and the oculars are convex lenses (Figure 1 - 1). When a specimen "AB" is put under the objective and focused, a magnified upside-down virtual image "ꓭV" will form on our retinas (Figure 1 - 2).

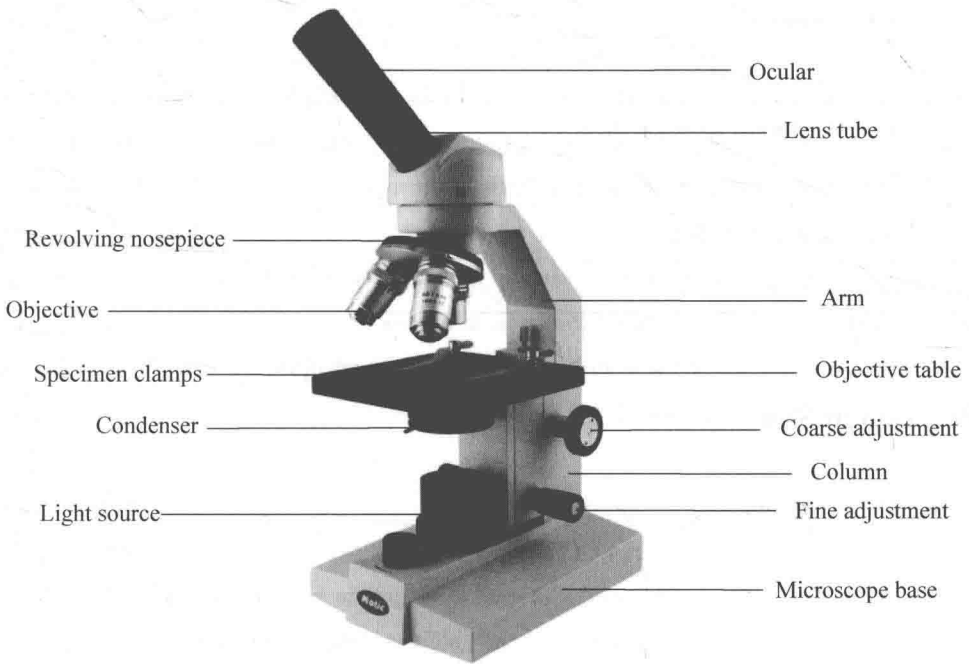


Figure 1 - 1 Structure of Microscope

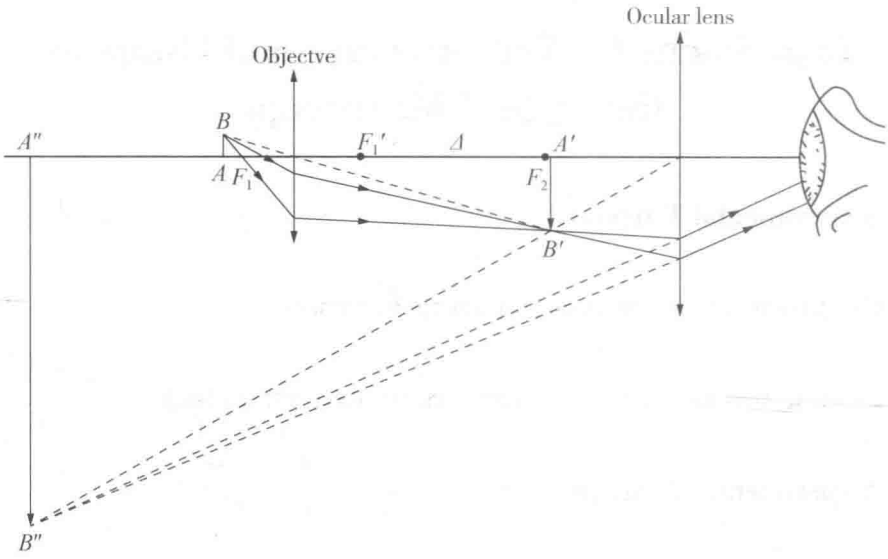


Figure 1 – 2 Microscope Imaging Principle

Table 1 – 1 The Numbers of Equipments and Reagents

Equipments and Reagents	Numbers for Each Group
1. Optical microscopes	4
2. Letter slides	4
3. Crossing hair slides	4
4. Toad blood smear specimen slides	4
5. Cedar oil bottle	1
6. Oil remover reagent bottle	1
7. Lens tissue box	1

Ⅲ. Group Size

4 students per group.

Ⅳ. Experimental Procedures

1. Structures and function of an optical microscope

(1) Mechanical parts.

①Microscope base: bottom of a microscope used for supporting and stabilizing.

②Column: a connecting structure between the base and the arm.

③Arm: an arched columnar structure between the column and the tube, a holding part when removing a microscope.

④Lens tube: a cylindrical structure used for holding an ocular. With double oculars, there are double lens tubes.

⑤Revolving nosepiece: a disc-like structure under the lens tubes and above the objective table. It has 4 circular holes used for fixing different objectives.

⑥Objective table: a small platform with two specimen clamps for fixing specimen slides located under the objective lens. Light emitting from the light source underneath illuminates the clamped specimen through the central hole of the objective table.

⑦Focalizer: an apparatus for focusing. It includes coarse adjustment and fine adjustment. The coarse adjustment can ascend or descend the objective table observably when focusing, while the fine adjustment can only ascend or descend the objective table on a much smaller scale.

(2) Optical parts.

①Ocular: which is installed on top of a lens tube, could magnify the image further. The most common ocular enlarges tenfold, marked as 10 ×.

②Objective: which is installed on the revolving nosepiece, facing the objective table. Each microscope generally has 4 objectives with different magnifications, 4 ×, 10 ×, 40 × and 100 ×, respectively. The objective is the most important optical part of a microscope, whose magnification factor decides the resolution. The 4 × and 10 × lens are usually called low power lens, the 40 × lens are called high power lens, and the 100 × lens are oil lens (need to be immersed into ceder oil when using).

③Condenser: which is installed under the objective table, a magnifying glass with an adjustable aperture. Its functions include collecting the light irradiating from light source and converging it on the specimen slide through the central hole of the objective table. Its adjustable aperture controls how much light passes through the specimen slide.

2. Method of using an optical microscope

(1) How to use a low power lens.

①Adjust the light intensity: there are two ways to adjust the light intensity. One is to control the power of the lamp in light source; the other way is to turn up or turn down the aperture of the condenser, either ascend or descend the condenser.

②Fix the specimen slide: put the specimen slide on the objective table and fix it with both specimen clamps. Make sure the coverslip of the specimen slide is facing upward, and the target tissue you are observing is just above the central hole of the objective table.

③Focusing: Working distance of objective — the small gap between the bottom of an objective and a specimen when in focus.

Objectives with different magnifications also vary in length. When one objective on a microscope is in focus, switch to another objective, it's also in focus. All objectives are made in different lengths just for sharing the same parfocal distance. (objective length + working distance = parfocal distance, Figure 1 - 3)

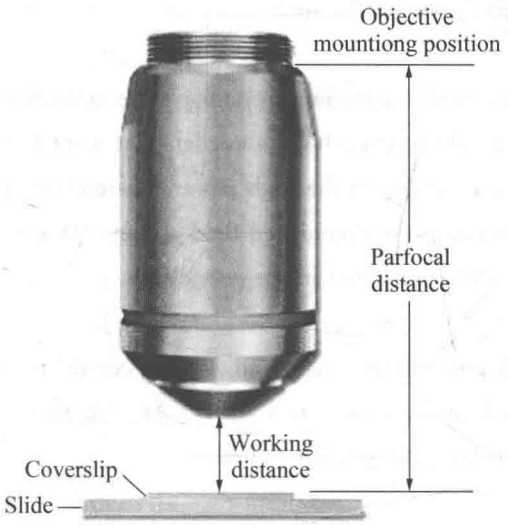


Figure 1 – 3 Objective Working and Parfocal Distance

The objective with the smallest amplification factor is the shortest one, and has the longest working distance. For our microscopes, the longest working distance of the shortest 4 × objective is 13 mm. Other working distances of objectives are 6.5 mm of 10 × objective, 0.48 mm of 40 × objective, and 0.23 mm of 100 × objective.

When focusing, we always start from the shortest low power lens, the 4 × objective, because it has the largest working distance and widest field of view.

After fixing the specimen slide on the objective table, with the target cell or tissue of specimen above the central hole, rotate the coarse adjustment to ascend the objective table until the gap between the bottom of the 4 × objective and the specimen a little bit smaller than the working distance of the objective.

Then rotate the coarse adjustment in the opposite direction to widen the gap between the bottom of the 4 × objective and the specimen. At the same time observe the specimen through oculars. You may get a clear image at the working distance.

Turn the knobs beneath the objective table to shift the clamped specimen back and forth, left and right, when probing the specimen. You may switch to 10 × objective in order to get a larger image.

(2) How to use high power lens.

①After focusing the low power lens, be sure to move the target tissue of the specimen to the center of the visual field because high power lens has a smaller field of view. Rotate the revolving nosepiece to switch to the high power lens and increase the power of the lamp for reduced light due to the diminished field of view. Observe through the oculars and turn the fine adjustment until the image gets clear.

②When focusing high power lens, coarse adjustment should not be used because the working distance of high power lens is really tiny. Any big shift of objective table will increase the risk of crushing the specimen slide.

When observing unstained cells, descend the condenser or/and turn down the aperture of the condenser, in order to increase contrast and get a clearer image.

(3) How to use oil lens.

①After focusing the high power lens, shift the target tissue of the specimen to the center of the visual field, and increase the power of the lamp a little bit.

②Rotate the revolving nosepiece a little to expose the sheltered target part of the specimen and put a drop of cedar oil on top of coverslip of the specimen. Then switch to the oil lens and make sure the bottom of oil lens immerged into the oil drop.

③Turn the fine adjustment slowly while observing through the oculars until you get a clear image. If you have difficulties in focusing, you may first ascend the objective table carefully to the terminal of oil lens by turning the fine adjustment. Attentively check in case the specimen slide breaks. Then turn the fine adjustment in the opposite direction while you observe through the oculars, until you get a clear image.

④When you have finished observing the oil lens, the ceder oil on the oil lens and the specimen must be wiped off instantly. Take out three pieces of lens tissue from its box, stack them together and fold in the middle. Then drip a drop of oil remover reagent onto the tissue stack and wipe the oil lens in one direction several times, wiping off all the residual oil. After that, wipe the oil lens with a stack of dry lens tissue to remove any

remaining oil remover reagent. Do the same thing to clean the specimen slide.

(4) How to use a double ocular optical microscope.

Double ocular optical microscopes provide you with two fields of vision. Just like wearing a pair of glasses, you have to get used to it. There is an arched scale at the bottom of one ocular and a white tiny spot at the bottom of another ocular. When the white spot points at any certain number of the arched scale, then the number represents the central distance between the two oculars. If this number equals to your interpupillary width, you will get a merged enlarged view.

V. Observation and Records

(1) Put the letter slide on the objective table and fix it, focus and observe the letter. Then compare the image of the letter with the original letter on the slide. A microscope produces an upside-down virtual image.

(2) Toad blood smear specimen is usually dyed blue or purple by hematoxylin-eosin staining. Toad red blood cells and nuclei are elliptical, nuclei are dyed dark purple blue, while cytoplasms are dyed light pink purple. Observe this specimen with low power lens, high power lens and oil lens.

(3) Finish your experiment report and submit it before you are dismissed.

VI. Attention

(1) When using an optical microscope, start from the low power lens to high power lens, then to the oil lens.

(2) When fixing a specimen on the objective table, remember always to keep the coverslip upward.

(3) When one finishes using an oil lens, the user must clean it as well as the specimen slide immediately with lens tissue and oil remover reagent.

(4) Focus slowly, and attentively check the objective and the specimen to avoid crushing it the specimen.

(5) Keep your optical microscope safe and clean. Cover it with its hood after use.

Experiment 2 Cell Microscopic Measurement

Ⅰ. Experimental Purposes

(1) Master the principle and method of cell microscopic measurement.

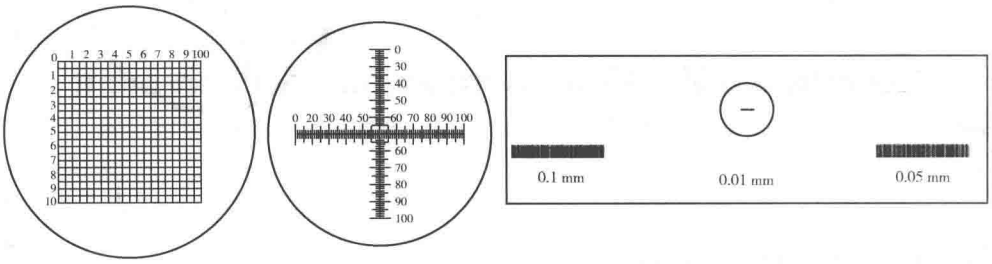
(2) Learn the method of determination and calculation of cell volume.

Ⅱ. Experimental Principle

Most cells are spheroidal or elliptical globular, with a diameter of about 20 micrometer (μm) for a common animal cell. A globular cell volume can be calculated according to the formula: $4/3\pi r^3$ ("r" represents radius), while an elliptical cell cubature formula is: $4/3\pi ab^2$ ("a" represents the long radius; "b" the short radius). We can work out the long radius and short radius according to measured diameters.

Micrometers including an ocular micrometer and an objective micrometer are often used to measure specimen cells. An ocular micrometer is a circular piece of glass with a graduated scale in the middle, while an objective micrometer is like a specimen slide, with a graduated scale in the middle too. An ocular micrometer can be put into one of the lens tube. The smallest per unit length of an objective micrometer is 0.01 mm (10 μm), and the smallest per unit length of an ocular micrometer depends on the amplification factor of the objective currently in use.

Before measuring a cell's diameter, we must determine the smallest per unit length of an ocular micrometer with the help of an objective micrometer under certain magnification (Figure 2 - 1).



(a) (b) (c)

Figure 2 – 1 Ocular Micrometer and Objective Micrometer

Table 2 – 1 The Number of Equipmants and Reagents

Equipments and Reagents	Numbers for Each Group
1. Optical microscopes	4
2. Toad blood smear specimen slides	4
3. Ocular micrometers	4
4. Objective micrometer	1
5. Cedar oil bottle	1
6. Oil remover reagent bottle	1
7. Lens tissue box	1

Ⅲ. Group Size

4 students per group.

Ⅳ. Experimental Procedures

1. Determine the smallest per unit length of an ocular micrometer

(1) Using low power lens.

Put an objective micrometer on the objective table and fix it with the specimen clamps. Ocular micrometers have already been placed into the lens tubes by technicians. After focusing the 4 × objective and getting a clear image of the objective micrometer, switch to the 10 × objective. Rotate the lens tube with the ocular micrometer inside to adjust

the micrometer's direction. Shift the objective table together with the objective micrometer until its zero point line overlapping with its counterpart of the ocular micrometer. Find another overlapping scale mark, and record the numbers of scale mark. Use the following formula to work out the smallest per unit length of the ocular micrometer in 10 × magnification.

$$\text{Per unit length of ocular micrometer } (\mu m) = \frac{\text{Overlapping scale mark's number of the objective micrometer}}{\text{Overlapping scale mark's number of the ocular micrometer}} \times 10\ \mu m$$

(2) Using high power lens.

Switch to high power lens. After further focusing by rotating the fine adjustment, shift the objective table together with the objective micrometer until its zero point line overlapping with its counterpart of the ocular micrometer. Find another overlapping scale mark, and record the numbers. Use the above formula to work out the smallest per unit length of ocular micrometer in 40 × magnification.

2. Measure two diameters of the toad red blood cells and the nuclei

Take down the objective micrometer and replace it with a toad blood smear specimen. Randomly choose 5 typical toad red blood cells and measure the long and short diameters of these cells along with their nuclei with the ocular micrometer in 40 × magnification. Record all of the numbers.

3. Work out the radii of cells and nuclei

Apply the above formula to convert all the measured diameter numbers into micrometers (μm). Then divide all of the micrometers into half to get the lengths of radii.

4. Calculate the average volumes of toad red blood cells and their nuclei

Calculate the average volumes of toad red blood cells and their nuclei according to the cubature formula: $V = 4/3\pi ab^2$ ("a" represents the long radius; "b" the short radius).

Finish your experiment report and keep your final result in scientific notation. Keep two significant figures after the decimal point. Submit it before you are dismissed.

V. Attention

(1) Be careful not to take diameter as radius.

(2) Don't take the unit numbers of ocular micrometer as real length.

(3) Be careful when manipulating the microscope. Don't crush the objective micrometer or other specimen slides.

(4) Don't disassemble the lens tube or ocular micrometer casually.