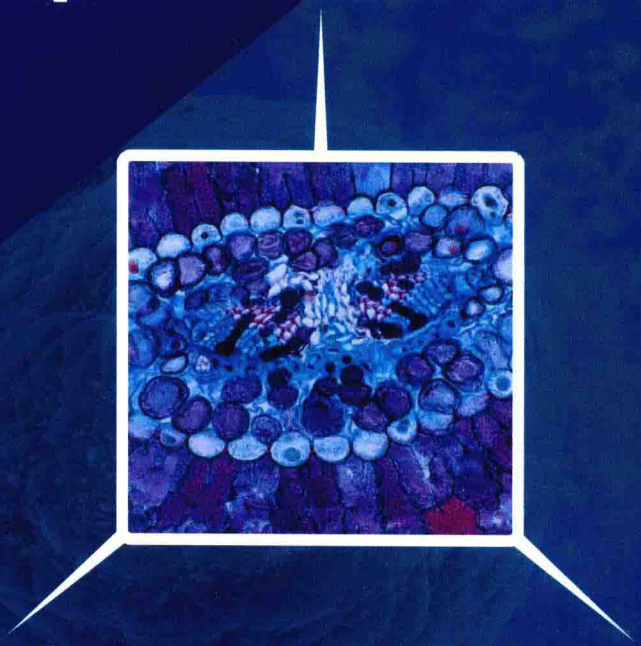
Practical Guidance for Cell Biology Experiments



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Practical Guidance for Cell Biology Experiments

(For Clinical Medicine MBBS Program)

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Introduction

This book is practical guidance for cell biology experiments. It is designed for the undergraduate medical students(Clinical Medicine MBBS Program) to enrich and strengthen their cell biology knowledge about cellular morphology, sub-cellular structure, cellular molecule and cellular function by performing relative practical experiments. This guidance includes four experimental sections as follows:

Section 1: Observation of Cellular Morphology and Measurement

Section 2: Observation of Sub-cellular Structure

Section 3: Cellular Molecular Test

Section 4: Cellular Function Test

These persons are involved in preparing this book: Ken Cheng(Sun Yat-Sen University) and Linzhu Wu, Junyu Cen, Ang Lu (Huazhong University of Science and Technology).

Contents

Usage of microscope

Microscope is equipment which can magnify the sample 16 *to* 100000 *times of the micro structures of specimens that cannot be viewed under natural conditions or with the naked eye.*

1. Microscope types

There are various types of microscopes used to observe specimen morphology and structures, such as optical or light microscope, fluorescence microscope, scanning electron microscope, transmission electron microscope (Fig. 1) and so on. The most common type of microscopes is optical microscope, which was first developed in the 16th century, using magnifying lenses and light to view specimens. By the usage of microscope cell was first described in 1665 by Robert Hooke in his book, *Micrographia*. Antonie van Leeuwenhoek discovered bacteria, spermatozoa, muscular fiber and some parts of cell structures such as cellular vacuole, by the optical microscope made by himself. Eric Betzig, William Moerner and Stefan Hell, the Nobel Prize winners in Chemistry in 2014, have developed super-resolved fluorescence microscopy to observe nano-dimensional structures and materials.

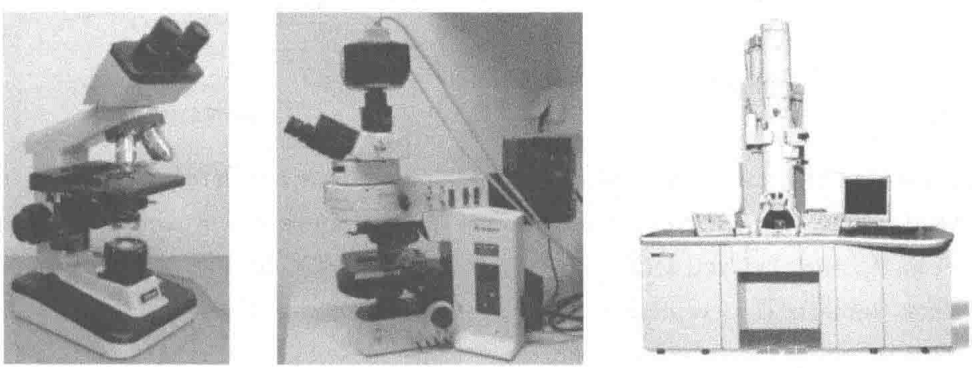


Fig. 1 From left to right: optical microscope, fluorescence microscope (Olympus BX61), transmission electron microscope

Other types of microscopes, such as confocal microscope, dark field microscope, photon scanning tunneling microscope, quantum microscope etc. are also used. Here we focus on the usage of optical microscope. It is necessary for students to be familiar with the structure of the microscope and correctly operate the microscope.

2. Basics for microscope use

1) **Carrying microscope:** Firstly clear your work space, and grasp the arm of the optical microscope firmly by one hand and lift its base with the other hand. Then set up the optical microscope on a level, clear desk.

2) **Cleaning lenses:** Only lens paper must be used for this task. Firstly keep the objective lens and ocular lens moist by breathing on them lightly and then clean them by lens paper. If the view of optical microscope still remains foggy, you can ask for assistance from the teacher or laboratory technician.

3) Always firstly observe the specimen at the low magnification objective (4×) by optical microscope.

4) Initially focus by lowering the stage to the focal point with the coarse focus knob. Never raise the stage using the coarse focus knob during focusing, otherwise, the objective lens may ram the specimen, which can damage both.

5) Use the fine focus knob to focus at high of objective lens. If you totally lose focus, return to a low of objective lens to find the focal point.

6) Do not use the 100× objective lens unless you have received specific instructions for it, since it is an oil immersion lens which needs oil to lubricate it during usage.

3. Common structures of compound optical microscope

The critical structures of two common optical microscopes are shown in Fig. 2.

The compound optical microscope has two systems of lenses for high magnification, namely:

- Eyepiece or ocular lens.
- Objective lens.

Main parts of the optical microscope and their functions are listed as below:

- **Ocular lens**, also called eyepiece, is closest to the eye when using microscope and it is the part at the top that you look through. It is usually 4× or 10× power(Fig. 3).
- **Tube** connects the eyepiece to the objective lenses(Fig. 3).
- **Arm** supports the tube and connects it to the base.
- **Nosepiece or Turret** is the part that holds two or more objective lenses and can be rotated to easily change the power(Fig. 4).
- **Objective lenses** have 3 or 4 powers consisting of 4×, 10×, 40× and 100× powers.
- **Base** is the bottom of the microscope used for support(Fig. 5).
- **Illuminator** is a steady light source used in place of a mirror(Fig. 5).
- **Stage** is the flat platform where you place your slides. Stage clips hold the specimen in place. A mechanical stage, allows you to move the specimen around by turning two knobs. One knob is used to move the specimen between left and right, while the other is

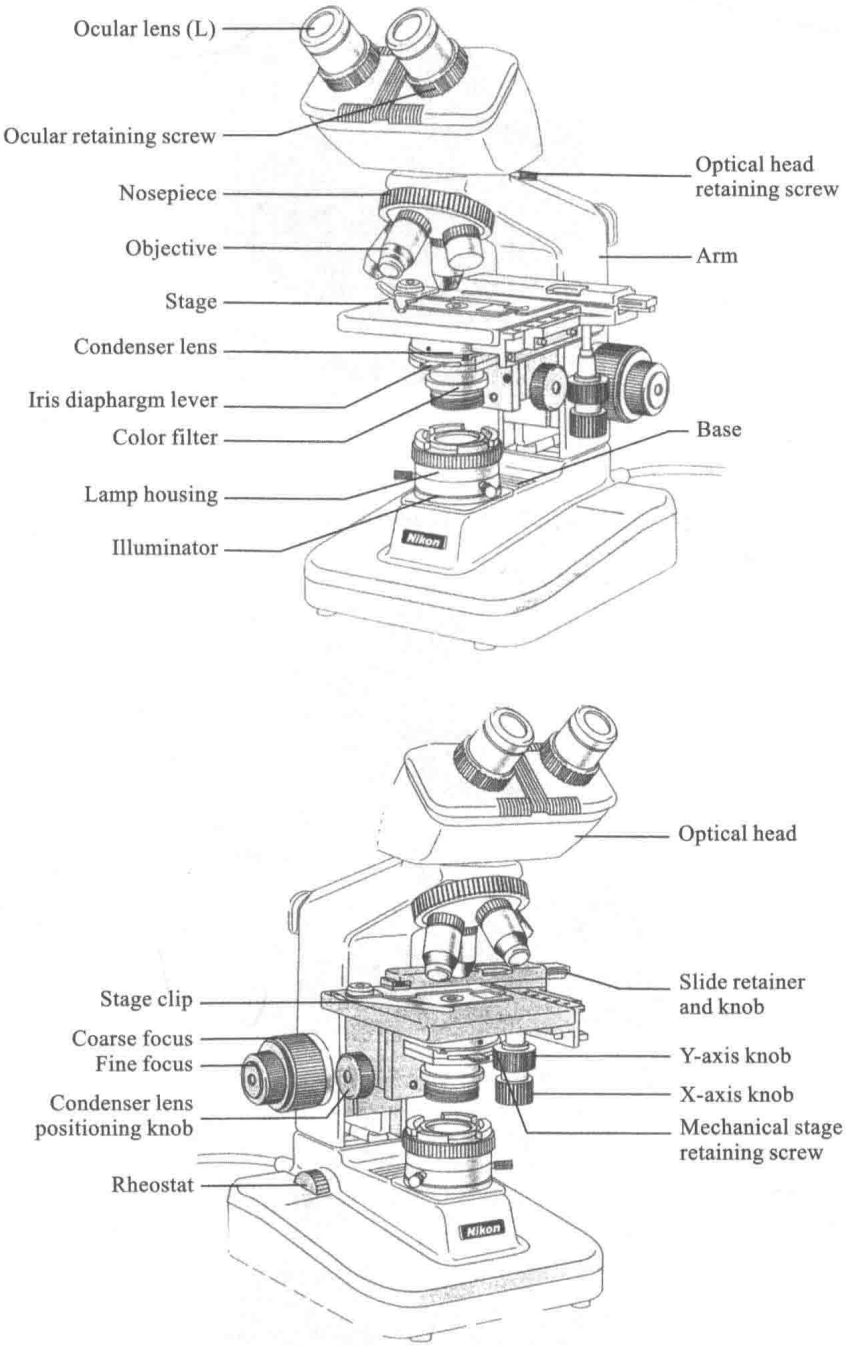


Fig. 2 Diagrams of common microscope structures

used to move it between up and down(Fig. 5).

- **Condenser lens** is to focus the light onto the specimen. Condenser lenses are most useful at the highest powers, namely 400× and above(Fig. 5).

- **Diaphragm or Iris** is under the stage with different sized holes. It is used to adjust the optical intensity and spot size as the light is projected upward into the specimen(Fig. 5).

Fig. 3 Microscope ocular lens and tube

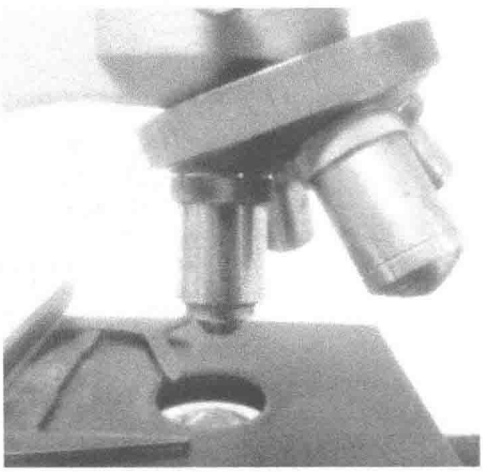


Fig. 4 Microscope turret and objective lenses

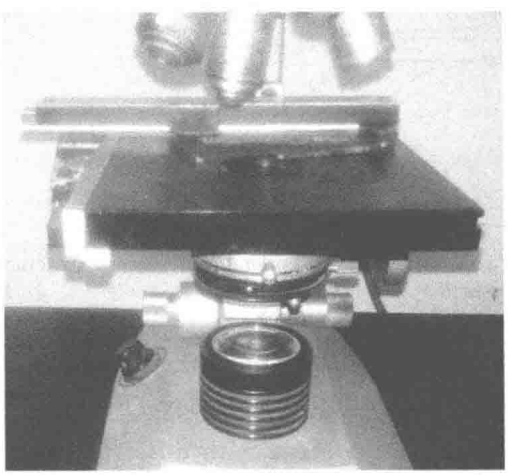


Fig. 5 From the bottom to up: base, illuminator, condenser lens, iris and stage

Slide viewing

1. Prepare a slide or use a pre-prepared slide with samples to be observed by optical microscope.

2. Clean all ocular and objective lenses before usage.

3. Rotate the coarse adjustment knob carefully to obtain the maximum working distance.

4. Place the slide on the stage. Make sure that the slide should fit into the slide holder and rotate the stage adjustment knob to move the slide forward or backward and left or right to the opening region in the stage.

5. Rotate the lower objective lens (such as 10×) to close the slide and rotate the coarse adjustment knob to obtain the minimum working distance.

6. Look through the ocular lens while adjusting the light with the iris or diaphragm lever if necessary. Turn the coarse adjustment knob very slowly and carefully until the view is becoming into focus. Then turn the fine adjustment knob more slowly and carefully to sharpen the focus.

7. Rotate the stage adjustment knob slowly to move the view region on the slide until finding the idea area, observe carefully and record them correctly. The examined object should be in the center of the field.

8. Change the high power objective (such as 40×) into place by rotating and turn the fine adjustment knob to sharpen the focus. **Be careful: Do not use the coarse adjustment knob at this stage, which will crack the slide easily.** The light can be adjusted again using the iris or diaphragm lever to obtain more brighter views if necessary. Adjust the view region on the slide until find the idea area, examine more closely and record results correctly.

9. If necessary for higher magnificence, rotate the high power objective lens (such as 100×) halfway to the next position, place a drop of immersion oil on the slide, and then rotate the oil immersion objective lens into place. The objective lens should be immersed in the oil on the slide. Again, use the fine adjustment knob to sharpen the focus carefully and adjust the light by the iris or diaphragm lever if necessary. Then adjust the view region on the slide until finding the idea area, observe carefully and record results correctly.

10. After finishing observing, turn the coarse adjustment knob to maximize the working distance and remove the slide from the stage. Clean the microscope, return every part to the original condition and store it in place.

Microscope cleaning techniques

The following techniques and procedures should be mastered for cleaning the microscope so as to keep the instrument using conveniently during the experiment.

1. Turn off the light before cleaning the microscope.

2. Turn the coarse adjustment knob to obtain the maximum working distance and remove the slide from the stage.

3. Clean all the lenses by fine lens paper ONLY. Clean ocular lens first, and then clean the objectives lens. If necessary, breathing on the lenses prior to using the lens paper may help to clean the lenses easier.

4. Clean any oil off on the stage by paper towels.

5. Rotate the objective lens into place and turn the coarse adjustment knob to obtain the minimum working distance.

6. Return the microscope to the appropriate storage area.

Slide cleaning techniques

The glass, microscope slides should also be cleaned and dried before placing a specimen on it since any small foreign debris might affect the observation of samples.

1. Hold the slide from its ends by the fingers.

2. Clean the slide by detergent liquid carefully with one finger to remove any foreign debris.

3. Wash the slide under running water and clean many times until no trace of the detergent is left, and then rinse the slide with distilled water.

4. Dry the slide by placing it between two towel papers, or place it into alcohol solution to store them until used. **Be sure to hold a clean slide on its end. Never touch the middle part of a slide.**

Section 1 Observation of Cellular Morphology and Measurement

Experiment 1 Observing Cells under Optical Microscope

1.1 Objectives

1. Familiarize with the structure and function of each part of the microscope.
2. Familiarize with basic method for the usage of microscope.
3. Find out cells under the microscope.
4. Observe various cell morphologies.
5. Present the observations with correct biological diagram techniques.

1.2 Introductions

Observation of the cell morphology and cell type is basic knowledge. Cell morphology generally is one of principles for cell classification. Basically there are two kinds of cells, prokaryotic cell such as bacteria and eukaryotic cell including plant and animal cells(Fig. 6).

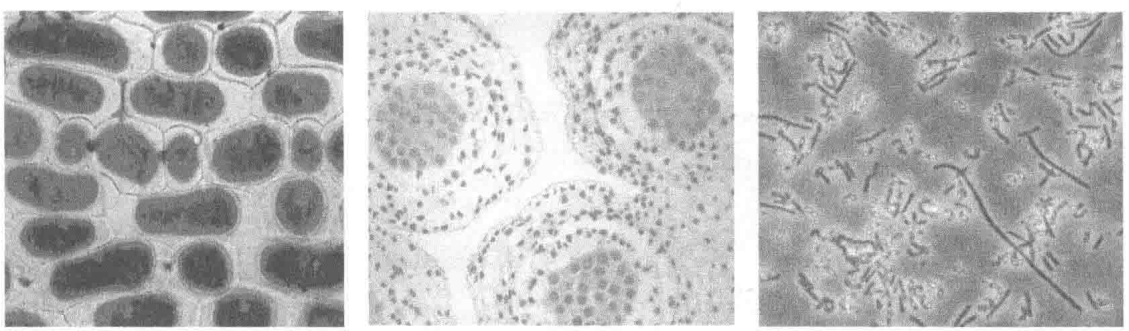


Fig. 6 From left to right: plant cells, animal cells and bacterial cells

Knowing cell morphology and cell type is helpful to understand the disease development. The changes of cellular morphology such as granularity around the nucleus, detachment of the cells from the substrate, and cytoplasmic vacuolation are following with pathological process. Some typical mammalian cell morphological characteristics are listed in Fig. 7 and Fig. 8.

- **Fibroblast cells** can be bipolar or multi-polar and have elongated shapes.
- **Epithelial cells** are polygonal in shape with more regular dimensions. They tend to grow in discrete patches within a substrate.
- **Lymphoblast cells** are spherical in shape and usually grown in suspension without attaching to the surfaces.
- **Neuronal cells** are further divided into two general morphologies, namely type 1 which have long axons and type 2 which have no axons.

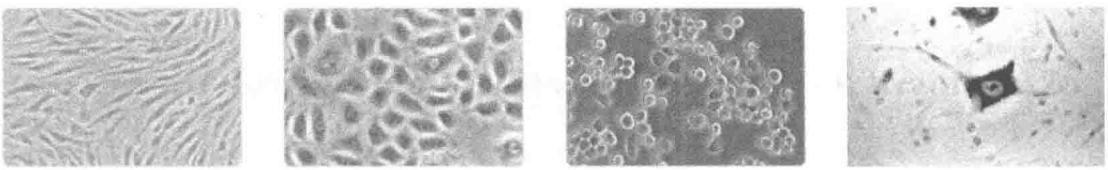


Fig. 7 From left to right: fibroblast, epithelial, lymphoblast and neuronal cells

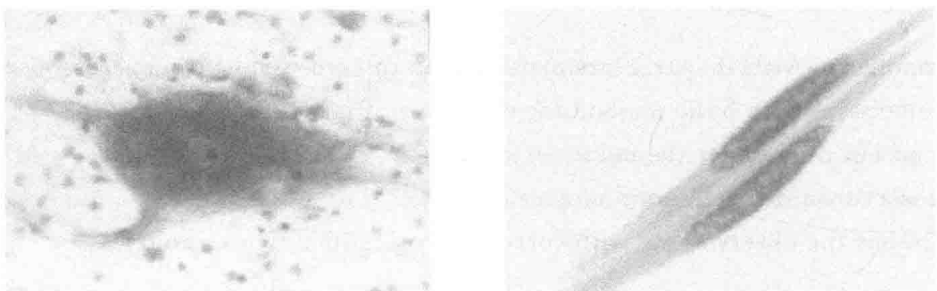


Fig. 8 From left to right: neuron and smooth muscle cell

In addition, observation of pathogens such as bacteria and viruses is also important. The bacteria cells are prokaryotic with no nucleus enveloped by nuclear membrane compared to eukaryote(Fig. 9). Definition of cell types is a vital diagnostic tool as well as evaluation of treatment plan for diseases.

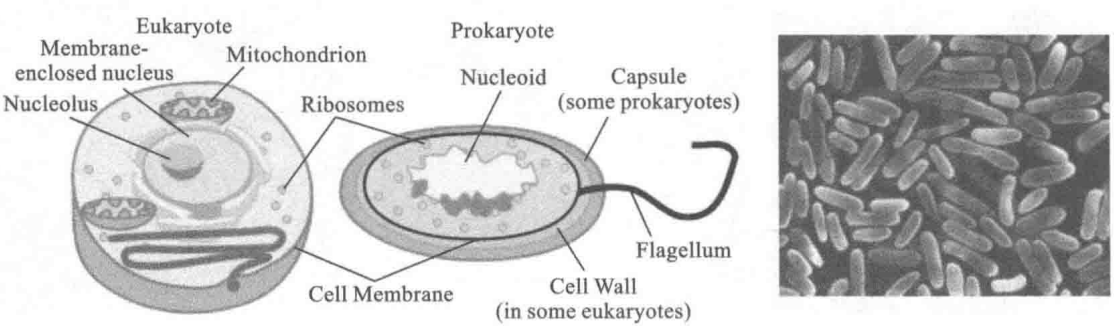


Fig. 9 From left to right: eukaryote, prokaryote and *E. coli*
(from http://en.wikipedia.org/wiki/Prokaryote and http://image.baidu.com/)

1.3 Principles

A simple microscope composes of a lens or set of lenses including objective and ocular

lens to amplify a specimen. Lens with different magnification factors are used to obtain different amplification times. Commonly, microscope viewing range is from 10^{-9} to 10^{-3} m (Fig. 10).

Here we mainly introduce a compound optical microscope. This microscope makes use of a light source and lenses to enlarge specimens and makes them visible (Fig. 11). Light sources of optical microscope are visible light, laser or LED. The magnification factor of optical microscope is the product of the powers of the ocular lens (eyepiece) and the objective lens. The maximum normal magnifications of the ocular and objective lenses are 10× and 100× respectively, giving a final magnification of 1000×. The amplified images can be recorded by hand-writing, or captured by digital cameras, CMOS or CCD detector.

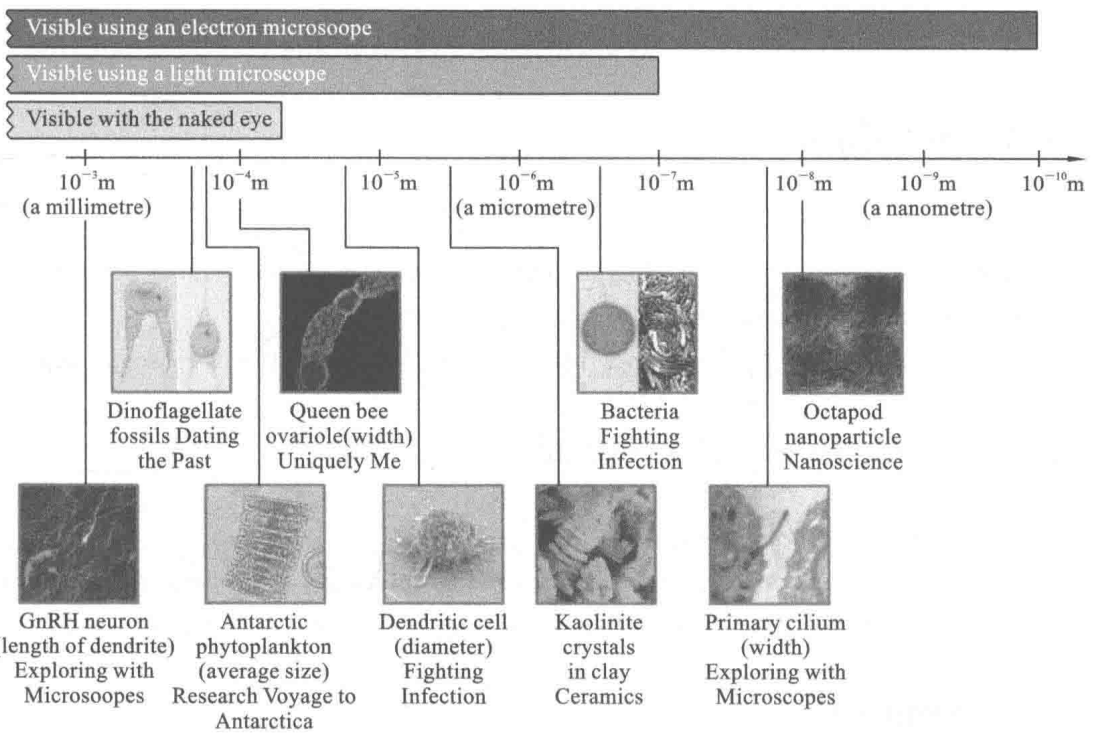


Fig. 10 Microscope viewing range

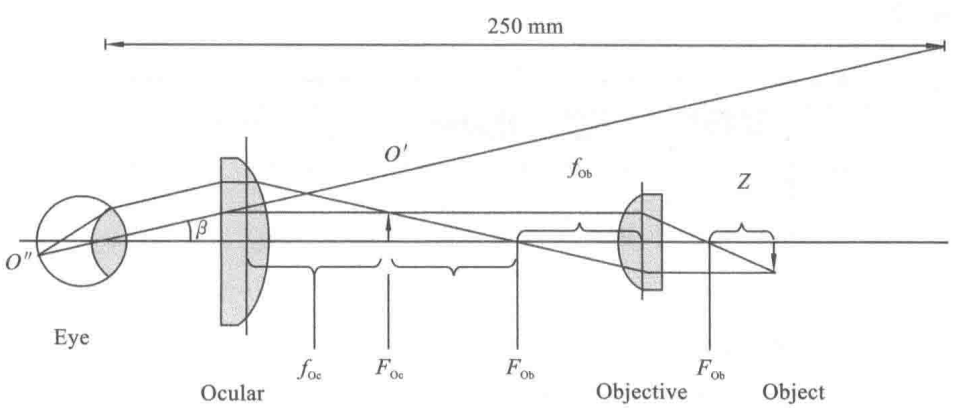


Fig. 11 Light pathway through a microscope

To increase the contrast between object to be observed and background, specimens are usually stained with different dyes(Fig. 12).

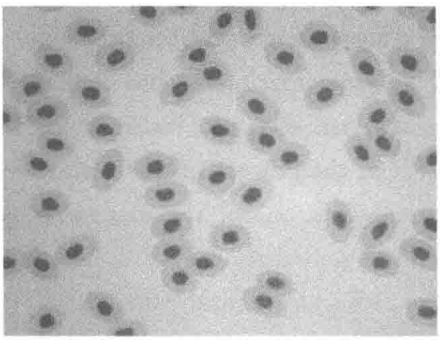


Fig. 12 Blood smear slide of chicken by Wright's staining (10×40)

1.4 References

1. Cell Morphology, Life Technology. https://www.lifetechnologies.com/cn/zh/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/sample-considerations/cell-morphology.html

2. History of the Light Microscope. http://www.history-of-the-microscope.org/

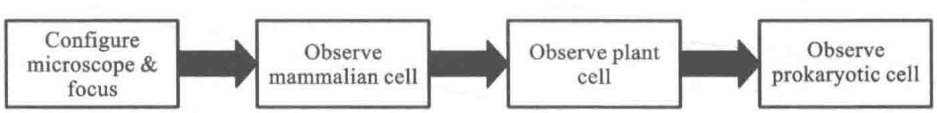
1.5 Apparatuses and materials

Apparatuses: optical microscope.

Materials: lens papers, pre-prepared colored wool slides, pre-prepared mammalian tissue cell slides, pre-prepared plant tissue cell slides, pre-prepared prokaryotic cell.

1.6 Protocols

Flow chart:



Procedure:

Focusing of microscope

1. Configure your microscope as per the instructions in the usage of microscope in this book. Be sure that it is stable on your desk, power on and the objective lens in the maximum working distance.

2. Select the pre-prepared wool slide sample.

3. Place the slide on the stage and adjust it accordingly using the stage adjustment knob so as to align it over the opening in the stage.

4. Rotate the objective lens into place and obtain the minimum working distance with the course adjustment knob.

5. Watch through the ocular lens, adjust the light with the iris lever, and then adjust the focus using the coarse adjustment knob until the image comes into a reasonably focused view. The fine adjustment knob can be used to sharpen the focus.

6. Further adjust the slide's position so as to obtain the part of the slide you wish to observe.

7. Observe the strands of wool in the slide and determine which strand is on top. Record what you observe on your data sheet and what strand of wool you believe to be on top.

8. Adjust the objective lens back to the maximum working distance once you have finished the whole experiment.

Cell observations

1. Select suitable mammalian cell, plant cell and prokaryotic cell slides to observe by the microscope.

2. Place one of the slides on the stage and bring it into focus as before.

3. Observe the celluar shapes, measure the cell size, identify organelles and their positions inside the cell. Be careful when observing any unique features of the cell.

4. Record all results on the data sheet and draft all of observation. This will be reproduced in your final report.

5. Repeat steps 1 to 4 to observe other cell slides.

1.7 Additional precautions

Strictly follow the instructions when using the microscope so as not to damage it.

1.8 Additional reading

Microscopy and Cell Cultures

History of the microscope

In the 1st century AD, the Romans experimented with different shapes of clear glass. They discovered that if you held one of these "lenses" over an object, the object would look larger. These early lenses were called magnifiers or burning glasses.

The first compound microscopes

The early microscopes, which were only magnifying glasses, had one power, usually about 6 ×-10 ×. Two Dutch spectacle makers, Zaccharias Janssen and his father Hans

started experimenting with these lenses. They put several lenses in a tube and made a very important discovery. The object near the end of the tube appeared to be greatly enlarged, much larger than any simple magnifying glass could achieve by itself.

Antonie van Leeuwenhoek became more involved in science and with his new improved microscope was able to see things that no man had ever seen before. He saw bacteria, yeast, blood cells and many tiny animals swimming about in a drop of water. People did not realize that magnification might reveal structures that had never been seen before. The idea that all life might be made up of tiny components unseen by the unaided eye was simply not even considered.

To increase the power of a single-lens microscope, the focal length has to be reduced. However, a reduction in focal length necessitates a reduction of the lens diameter, and after a point, the lens becomes difficult to see through.

To solve this problem, the compound microscope system was invented in the 17th century. This type of microscope incorporates more than one lens so that the image magnified by one lens can be further magnified by another.

Modern day microscopes

In recent times, the development of the microscope has slowed, since optical principles are well understood and to an extent, the optical limits have been reached. The majority of microscopes follow the same structural principles that describe monocular, mono-binocular and stereo-binocular microscopes.

Cell culture

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favourable artificial environment. The cells are removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation. They may be derived from a cell line or cell strain that has already been established.

Primary culture

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and developed under the appropriate conditions until they occupy all of the available substrate. At this stage, the cells have to be subcultured by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Cell line

After the first subculture, the primary culture becomes known as a cell line or subclone. Cell lines derived from primary cultures have a limited life span and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell strain

If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional