21世纪高等医学院校教材

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A Laboratory Manual of Histology & Embryology

组织学与胚胎学实验指南

（英文版）

附彩色插图

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内 容 简 介

双语教学是21世纪我国高等院校教学改革之大势所趋。本书就是为高等医学院校组织学与胚胎学课程编写的配套英文教材。全书包含实验指导和彩色图谱两个部分。实验指导部分介绍组织学与胚胎学各章节实验的目的、内容和观察方法，其中胚胎学部分还配有思考题，以帮助学生理解胚胎发生的变化过程。图谱部分配合实验指导，有近200幅照片，使学习过程形象、生动。

本书适用于医学院校各专业实验课教学和学生复习、自学。

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Preface

For many years the medical students in China have been used to accepting lectures in Chinese. The situation has been changed dramatically in recent years owing to the economic reform and China's opening to the outside world. There has already been a great boom in foreign language education in China. It may be said without much exaggeration that learning English has become fashionable in China now. Facing the fast developing advanced science and technology in the world one cannot join international competitions without mastering at least one foreign language. That is why Ministry of Education encourages introducing bilingual education in high education and hoping to give some courses in foreign languages gradually.

Histology and embryology are courses that medical students will meet in their first college year. We always say that a good start is half a success. That is why we have written this *Laboratory Manual of Histology and Embryology*, to meet the needs of first-year medical students in their courses and also to provide a valuable reference for postgraduates and junior faculties in their professional English training. It is our hope that the students who read this manual will be helped to improve their meeical English and be benefited in their future medical courses study.

This manual is not intended to be a Histology text. Many fine textbooks of Histology are already available. This manual is just designed as a laboratory aid and contains figures designed to make it easier for students to orient themselves and to locate and identify features of each kind of tissue. It is the authors' hope that this work might serve as a valuable laboratory aid for students attempting to study medicine in English and recognize the microscopic features of normal tissues and as a supplement to the many fine textbooks. We would be glad to receive any notes of errors, as well as of relevant important information that may have been missed.

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Guangzhou
December, 2002

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INTRODUCTION TO HISTOLOGY

USING THE MICROSCOPE

See Fig 1-1 for a picture of a microscope.

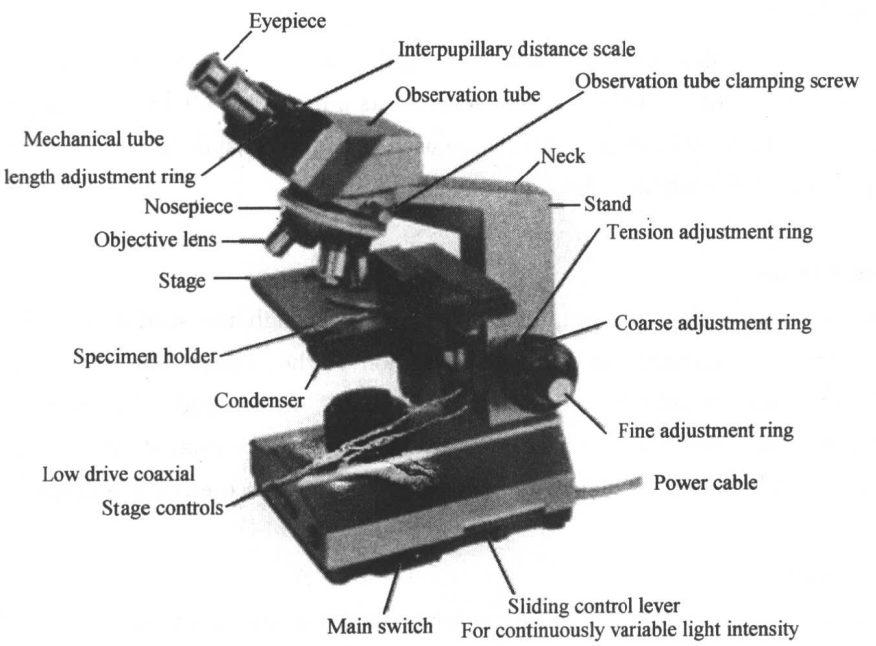

Fig 1-1 A microscope

MICROSCOPE COMPONENTS

Light Source

Light is provided by an incandescent globe on the base of the microscope and is filtered through a blue filter.

Iris Diaphragm

It is the aperture, which controls the diameter of the light beam. If the diaphragm is closed too much, the image becomes too dark. If it is left wide open, the image will become glary due to too much light interference. In both extremes, image quality is poor. The iris diaphragm is housed in the condenser.

Condenser Lens

The main purpose of the condenser is to focus or concentrate the available light onto the plane of the section (slide). The more light passing through the section, the better the resolution will be. This determines the amount of detail that can be seen. The condenser is capable of vertical adjustment to allow for the various thickness of slides.

Specimen Stage

The stage lies above the condenser and is a rigid, flat platform with an aperture through which the light can pass onto the section. Its purpose is to hold the glass slide bearing the section. Coaxial stage controls are used to move the slide in two directions to allow viewing of the whole slide.

Objective Lenses

The objective lenses collect the light that has passed through the section and along with the eyepiece, determine the final magnification of the image. The microscopes are usually equipped to hold 4 objectives (4×, 10×, 40×, 100×), which are housed in a revolving nosepiece. This allows the objectives to be rotated into the light path with ease. Changing the objectives requires adjustment in the iris diaphragm.

Focus Controls

A blurry or fuzzy image is brought into focus using the coarse and fine focus controls. At lower magnifications, i.e., lower objectives (4×, 10×), the coarse focus control can be used. At higher magnifications (20×, 40×), the fine focus control should be used.

Eyepiece

The eyepiece focuses the light into the eye and magnifies the image along with the objective lenses. On the student microscopes the eyepieces are of 10× magnification. The eyepiece is held in the observation tube.

PROCESSES OF OBSERVING THE SLIDE

It is a good idea to start with the **naked eyes** on the **lowest power objective** (4×) and then go up to the **higher powers** to see a feature magnified. Take a slide from the box and examine the section with your naked eyes (gross) to determine the shape and the color of the section. The eyepiece can be used as a **hand magnifier** to examine the histological slide. The eye lens of the eyepiece should face the slide (reverse the eyepiece). This scanning will give you a general idea about the major structures of the slide. When you put the slide on the stage, make sure it is **coverslip up**, otherwise the high objective lenses will crush the slide or you can never focus on the section through the thickness of the mounting slide with high-power objective. The next step is to **optimize the light**. A light source should have a wide dynamic range, to provide high intensity illumination at high magnifications, and lower intensities so that the user can view comfortably at low magnifications. Some microscopes have a built-in illuminator. If your microscope requires an external light source, make sure that the light is aimed at the center of the condenser. Adjust illumination so that the field is bright without hurting the eyes. If the condenser has selectable options, set it to bright field. Start with the aperture diaphragm stopped down (high contrast). You should see the light that comes up through the specimen, change brightness as you move the aperture diaphragm lever. **Start with the lowest magnification objective lens,** to home in on the specimen and/or the part of the specimen you wish to examine. Start with the specimen out of focus so that the stage and objective must be brought closer together. The first surface to come into focus as you bring stage and objective together is the top of the coverslip. Once you have found the specimen, adjust contrast and intensity of illumination, and move the slide around until you have a good area for viewing. **With a binocular microscope** you need to adjust the eyepiece separation. One of the eyepieces may be a telescope eyepiece. You can focus one eyepiece to match the other image. Look with the appropriate eye into the fixed eyepiece and focus with the microscope focus knob. Next, look into the adjustable eyepiece, and adjust the eyepiece, not the microscope. The most frequently used objective lens is the 10×. For very small protists and for details in prepared slides such as cell organelles or mitotic figures, you will need a higher magnification. Typical high magnification lenses are

40× and 100×. The latter is used exclusively with oil in order to improve resolution. Each time you go to a higher power objective, re-focus and re-center the specimen. With a low power objective you may have to cut down on illumination intensity. With a high power you need as much light as you can get.

CARE OF THE MICROSCOPE

- Hold a microscope firmly always by the stand. Never grab it by the eyepiece holder.
- Hold the plug (not the cable) when unplugging the illuminator.
- Since bulbs are expensive, and have a limited life, turn the illuminator off when you are done.
- Always make sure the stage and lenses are clean before putting away the microscope.
- NEVER use anything but good quality lens tissue on any optical surface, with appropriate lens cleaner or distilled water; organic solvents may separate or damage the lens elements or coatings.
- Cover the instrument with a dust jacket when not in use.
- Focus smoothly; do not try to speed through the focusing process or force anything.

HISTOTECHNIQUES

1. TISSUE COLLECTING

Tissues, whether biopsies, larger specimens removed at surgery or tissues from autopsy, should be collected freshly and fixed immediately. The size of the tissue block should be less than 1.2cm×0.5cm×0.2cm.

2. FIXATION

The purpose of fixation is to preserve tissues permanently in as life-like a state as possible. Fixation should be carried out as soon as possible after removal of the tissues (in the case of surgical pathology) or soon after death (with autopsy) to prevent autolysis. The quicker you get the tissue and fix it, the better. Artifact will be introduced by drying, so if tissue is left out, keep it moist with saline. The longer you wait, the more cellular organelles will be lost and the more nuclear shrinkage and artificial clumping will occur.

There is no perfect fixative, though formaldehyde comes the closest. Therefore, a variety of fixatives are available for use, depending on the type of tissue present and features to be demonstrated.

3. TISSUE PROCESSING

Once the tissue has been fixed, it must be processed into a form in which it can be made into thin microscopic sections. The usual way is to operate in paraffin, which is similar in density to tissue. The technique of getting tissue fixed into paraffin is called **tissue processing**. The main steps in this process are **dehydration** and **clearing**.

Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water in the tissues must be removed by dehydration. This is usually done with a series of alcohol solutions, say 70% to 95% to 100%.

The next step is called "clearing" and consists of removal of the dehydrator with a substance that will be miscible with the embedding medium (paraffin). The most common clearing agent is xylene. Finally, the tissue is infiltrated with the embedding agent, almost always in molten paraffin for three times. Paraffins of different melting point (52 – 54℃; 54 – 56℃; 56 – 58℃) can be purchased for various hardnesses.

Then the tissues must be manually put into the blocks by picking the tissues out of the cassette and pour molten paraffin over them. This "embedding" process is very important, because the tissues must be properly aligned, or oriented, in the block of paraffin.

4. SECTIONING

Once the tissues have been embedded, they must be cut into sections that can be placed on a slide. This is done with a microtome. The microtome is nothing more than a knife with a mechanism for advancing paraffin block by standard distances across it. A very sharp knife is the most important necessity for proper sectioning.

Microtomes have a mechanism for advancing the block across the knife. Usually this distance can be set from **6 to 8 microns**. for most paraffin embedded tissues

It is important to have a properly fixed and embedded block, or much artifact can be introduced in the sectioning. Common artifacts include tearing, ripping, "Venetian blinds", holes, folding, etc. Once sections are cut, they are floated on a warm water bath that helps remove wrinkles. Then they are picked up and placed on a glass microscopic slide.

Then place the glass slides in a warm oven for about 15 minutes to help the section adhere to the slide. If the heat might harm such things as antigens for immunostaining, then this step can be bypassed and use glue-coated slides instead to pick up the sections.

5. STAINING

The embedding process must be reversed in order to get the paraffin wax out of the tissue and allow water-soluble dyes to penetrate the sections. Therefore, before any

staining can be done, the slides are "deparaffinized" by running them through xylene (or substitutes) to alcohols and to water. No stains can be done on tissues containing paraffin.

The staining process makes use of a variety of dyes chosen by their capability to stain various cellular components of tissue. The routine stain is with **hematoxylin** and **eosin** (**HE**).

Hematoxylin, a basic dye, binds to acidic components of a tissue, which are thus said to be "**basophilic**." The color of the stained structures depends on the mordant used to make the hematoxylin dye bind to the molecules of the tissue. Potassium alum, the most common mordant, gives the dye a blue to purple color.

Eosin, an acidic dye, binds to cytoplasmic components of the cell, which are thus said to be "**acidophilic**". The structures stained by eosin are typically colored pink to red.

Other stains are referred to as "special stains" because they are employed in specific situations according to the diagnostic need.

6. COVERSLIPPING

The stained section on the slide must be covered with a thin piece glass to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years. The stained slide is taken through a series of alcohol solutions to remove the water, then through clearing agents to a point at which a permanent resinous substance beneath the glass coverslip can be placed over the section.

ARTIFACTS IN HISTOLOGICAL SECTIONS

A number of artifacts that appear in stained slides may result from improper fixation, from the type of fixative, from poor dehydration and paraffin infiltration, improper reagents, and poor microtome sectioning.

Tissues that are insufficiently dehydrated prior to clearing and infiltration with paraffin wax will be hard to section on the microtome, with tearing artifacts and holes in the sections. Tissue processor cycles should allow sufficient time for dehydration, and final ethanol dehydrant solution should be at 100% concentration.

Though alcohols such as ethanol make excellent fixatives for cytological smears, they tend to make tissue sections brittle, resulting in microtome sectioning artifacts with chattering and a "Venetian blind" appearance.

Bubbles under the coverslip may form when the mounting media is too thin, and as it dries air is sucked in under the coverslip. Contamination of clearing agents or coverslipping media may also produce a bubbled appearance under the microscope.