全国高等医药院校药学类实验教材

生药学实验

（第二版）

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副主编　贾　英

中国医药科技出版社

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内 容 提 要

本书为全国高等医药院校药学类实验教材，依据高等医药院校生药学教学大纲编写，为适应教育国际化的要求，增加了英文对照内容，以便于学生在阅读英文文献、撰写英文论文时参考。全书分为实验技术和实验内容两部分。在实验技术部分中主要论述了生药学实验中常用的显微、理化鉴别及含量测定的方法和注意事项；在实验内容部分为了让学生更好地掌握生药鉴别的共性，按照药用部位分类收载了20个实验，基本涵盖了生药学研究的各个方面，尤其是加入了开发性实验（药材标准的制定）、自我测试实验（未知生药粉末的鉴别）以及应用本领域新技术的实验（生药川芎的HPLC指纹图谱分析、高特异性PCR法鉴定生药蕲蛇），同时还设置了药用植物园和标本室的实习，因此具有很好的适教性和较高的参考价值。

本书可供高等医药院校相关专业实验教学使用，也可供行业培训使用。

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第二版前言

本书是高等医药院校药学、中药学专业本科专用实验教材。

本书依据高等医药院校生药学教学大纲编写，为适应教育国际化的要求，增加了英文对照内容，以便于学生在阅读外文资料、撰写英文论文时参考使用。

全书分为实验技术和实验内容两部分。在实验技术部分中主要论述了生药学实验中常用的显微、理化鉴别及含量测定的方法和注意事项。实验内容部分中按药用部位分类收载了20个实验，基本涵盖了所有生药学研究的内容，尤其是加入了开放性实验（实验十七：药材标准的制定）和自我测试实验（实验十六：未知生药粉末的鉴定），力求改变学生照单抓药的实验习惯，增强自主实验的能力。为适应生药学发展的需要，本书还收录了生药的指纹图谱分析（实验十八）和用DNA分子标记技术鉴别来自不同产地的生药（实验十九），并在实验中详细注明了原理、方法和程序。如果没有条件开展这些实验，也可用教师或图像演示实验代替。为弥补某些院校药学专业不设药用植物学课程的缺憾，本书加设了在药用植物园和标本室的实习（实验二十）。在再版中我们总结了原版教材在应用过程中存在的问题，并紧随科学发展增加了新研究成果，主要进行了如下修订：总论中加入了质谱联用的方法，实验八中增添了新的内容，实验十一中改为动物类生药鹿茸的鉴定，实验十二中增添了蟾酥中脂蟾毒配基、华蟾酥毒基的HPLC含量测定方法，实验十五中增加了多糖类和黄酮类的鉴别方法，实验十八中增加了川芎中阿魏酸的含量测定方法，实验十九改为高特异性PCR法鉴定生药蕲蛇，并调整了原书中部分实验内容的章节顺序，如原书中实验五的薄荷的鉴定，因其药用部位为全草类，调整至实验九。

本书是由沈阳药科大学生药学教研室教师编写的。中文部分第二章中实验一、二、八、十一由韩娜副教授编写；实验五、六、七及实验九由贾英副教授编写；实验十、十二、十八、十九由王东副教授编写；实验三、十三、十四由刘志惠讲师编写；实验十五、十六由代英辉讲师编写，其余部分由殷军教授编写并统编全书，贾英副教授协助完成正文部分的校对工作；全文的英文翻译由殷军教授完成，韩娜副教授、赵旭讲师协助了部分英文的统编工作。日本富山医科药科大学外籍教师Faisal Haider先生和美国哥伦比亚大学的Nitesh sood先生对本书的英文内容进行了校订，在此表示谢意。

在编写过程中，由于时间仓促，水平有限，难免存在错误和疏漏之处，敬请批评指正。

编者

2014年1月

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目 录

实验室规则

1. 遵守实验时间，不得无故缺席或迟到、早退，如中途有事离开实验室，必须征得老师同意。

2. 每次实验内容事先应充分预习，并备好应携带的物品及有关参考资料（包括实验指导及教材）。

3. 爱护实验室内的一切公共财物。使用显微镜必须严格遵守操作规程，不得随意拆动。每次使用完毕要擦拭干净，妥善放置。如有损坏，应及时主动报告，并明确责任。

4. 爱护实验室内所准备的标本、样品（药材、显微切片等），观察后必须归还，不得损坏或私自携带出室外。

5. 实验室内应保持安静，严禁吸烟、随便说笑。

6. 有机溶剂、腐蚀性液体必须倒在废液缸内，不得倒入下水道。

7. 取用公共仪器、试剂、药材应及时放回原位，不得移到个人台面上使用。

8. 实验过程中必须遵守防火和防中毒的一切规则，杜绝一切可能发生的危险隐患。

9. 实验结束后，桌面清洗干净，洗净仪器、用具，归还原位。将报告交给指导老师检查后，方可离开实验室。

Regulations of Laboratory

1. Be punctual, do not be absent, late or leave early without reasons. If you want to leave the laboratory during the experiment, you must get the teacher's permission.

2. Thoroughly preview the contents of experiment in advance and get ready for the materials and reference books requested (including experiment guidance and textbook).

3. Take care of all the materials in the laboratory. When using the microscope, you must comply with the operating regulations strictly, and should handle the microscope carefully. Always after using, make it clean and place it correctly. If it is damaged, inform your teacher immediately and make yourself clear.

4. Take care of the specimen, showpiece (medicinal materials, slices, etc), and return after observation. Do not make any damage or take it out.

5. Be quiet and do not speak or laugh loudly. Smoking is forbidden.

6. Pour organic solvent and caustic liquid into waste jar. Do not pour it into drains.

7. Return the public apparatus, reagents andmaterials to the originl place; do not move them to your individual table.

8. Obey all the fireproofing and toxicosis rules during the experiment to stop all dangerous hidden troubles.

9. Once the experiment is over, clean up all the used tools, including your tabletop, instruments and appliance and put them back. You should not leave until you handover to your supervisor your experimental report.

第一章　实验技术

生药的鉴别包括基原、性状、显微、理化等四大传统鉴别方法，其中技术要求较高的是生药的显微和理化鉴定。生药学实验的重点也是指导学生掌握生药的显微和理化鉴定的方法。生药的显微鉴定主要涉及到显微镜的使用和生药显微标本的制作。生药的理化鉴定主要包括生药中主要化学成分的定性分析、微量升华法分析、荧光分析法分析、分光光度法和色谱法分析。下面就生药学实验中常用到的显微镜的使用、生药显微标本的制作、显微绘图法、显微化学鉴定、微量升华法、荧光法、分光光度法、色谱法及质谱联用技术做详细论述。

Chapter One Experimental Techniques

To identify crude drug usually four traditional methods are used, which are original plant, description of morphous, microscopic characteristics, physical and chemical identifying methods. Among them the later two methods need more technological skills. The point of our experiments is to guide students to learn microscopic characteristics and physical and chemical identifying methods. The microscopic identification mainly refers to identifying crude drugs by using a microscope and preparation of specimen. Physical and chemical identifying methods mainly include qualitative assay, microsublimation, fluorometric analysis, spectrophotometry and chromatography of main chemical compositions in crude drugs. The following are detailed discussion about usage of microscope, preparation of specimens, microscopic chartography, microscopic chemical identification, microsublimation, fluorometric method, spectrophotometry, chromatography and hyphenated HPLC – MS methods.

第一节　显微镜的构造及使用注意事项

一、显微镜的类型

显微镜是研究生物的细胞结构、组织特征和器官构造重要的且不可替代的仪器，它主要包括以下种类。

1. 光学显微镜　以可见光作光源，用玻璃制作透镜的显微镜。可分为单式显微镜和复式显微镜。复式显微镜结构复杂，至少由两组以上透镜组成，是植物形态解剖实验最常用的显微镜，其有效放大倍数可达1250倍，最高分辨力为0.2μm。除一般显微实验使用的普通生物显微镜外，重要的可供研究用的还有暗视野显微镜，相差显微镜

和荧光显微镜。

2. 电子显微镜 是使用电子束为光源的显微镜，它以特殊的电极和磁极作为透镜代替玻璃透镜，能分辨相距2Å（1 Å = 1/10000mm）左右的物体，放大倍数可达80万~120万倍，其分辨力比光学显微镜大1000倍，是观察超微结构的重要精密仪器。

二、显微镜的构造

显微镜的基本构造包括保证成像的光学系统和用以装置光学系统的机械部分，如图1-1。

1. 机械部分

（1）镜座 显微镜的底座，支持全部镜体，使显微镜放置稳固。

（2）镜柱 镜座上面直立的短柱，支持镜体上部的各部分。

（3）镜臂 弯曲如臂，下连镜柱，上连镜筒，为取放镜体时手握的部分。

（4）镜筒 为显微镜上部圆柱中空的长筒，其上端置目镜，下端与目镜转换器相连。转换器下的镜筒能保护成像的光路和亮度。

（5）物镜转换器 接于镜筒下端的圆盘，可自由转动。盘上有3~4个安装物镜的螺旋孔。当旋转转换器时，物镜即可固定在使用的位置上，保证物镜与目镜的光线合轴。

（6）载物台（镜台） 为放置玻片标本的平台，中央有一通光孔。上面装有机械移动器，一方面可固定玻片标本，同时可以向前后左右移动，便于观察，有的上面还装有游尺。

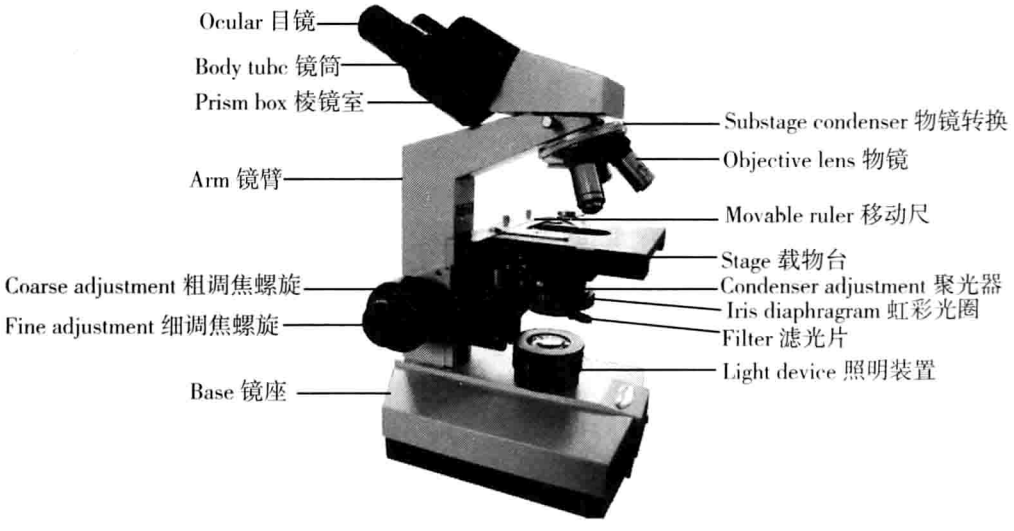

图1-1 双筒显微镜的构造

（7）调焦装置 用以调节物镜和标本之间的距离，使得到清晰的图像。在镜柱两侧有粗、细调焦螺旋各一对，旋转时可使载物台上升或下降，大的一对为粗调焦螺旋，旋转一周可使载物台移动2mm左右，小的一对为细调焦螺旋，旋转一周可使载物台移动约0.1mm。

2. 光学部分 由成像系统和照明系统组成。成像系统包括物镜和目镜，照明系统包括反光镜和聚光器。

（1）物镜　安装在镜筒下端的物镜转换器上，可分低倍、高倍和油浸物镜3种。物镜可将被检物体作第一次放大，一般其上均刻有放大倍数和数值孔径（N. A），即镜口率，如国产XSP—3C型显微镜有以下3种（表1-1）。

表1-1　XSP—3C型显微镜

物镜倍数	数值孔径（N. A）	工作距离（mm）
4×	0. 1	37. 5
10×	0. 25	7. 31
40×	0. 65	0. 63

工作距离是指物镜最下面透镜的表面与盖玻片上表面间的距离。物镜的放大倍数愈高，它的工作距离愈小（表1-1）。所以使用时要特别注意。

（2）目镜　安装在镜筒上端，可将物镜所成的像进一步放大。其上刻有放大倍数，如5×、10×、16×等。

（3）反光镜　是个圆形的两面镜。一面是平面镜，能反光；另一面是凹面镜，兼有反光和汇集光线的作用。反光镜具有转动关节，可作各种方向的翻转。将光线反射在聚光器上。

（4）聚光器　装于载物台下，由聚光镜和虹彩光圈等组成，它可将平行的光线汇集成束，集中于一点以增强被检物体的照明。

（5）虹彩光圈　装在聚光器内，拨动操作柄，可调节光圈大小，控制通光量。

三、显微镜的使用方法

1. 取镜和放镜　按固定编号从镜柜上取出显微镜。取镜时应右手握住镜臂，左手平托镜座，保持镜体直立，严禁用单手提着显微镜走动，防止螺旋脱扣。放置桌上时，一般应放在座位的左侧，距桌边约10～15cm处，以便观察和防止掉落。

2. 对光　一般可用窗口进入的散射光，避免用直射阳光；或用日光灯做光源。对光时先把低倍镜转到中央，对准载物台上的通光孔，然后用左眼或双眼从目镜向下注视，同时，转动反光镜，使镜面向着光源，光弱时可用凹面镜。当在镜筒内见到一个圆形而明亮的视野时，再利用虹彩光圈调节光的强度，使视野内的光线均匀而明亮。

3. 低倍镜的使用　观察任何标本，都必须先用低倍镜，因低倍镜的视野大，容易发现目标和确定要观察的部位。

（1）放置切片　降低载物台，把玻片标本放在载物台中央，使材料正对通光孔。然后用移动器固定住载玻片的两端。

（2）调整焦点　两眼从侧面注视物镜，并慢慢按顺时针方向转动粗调焦螺旋，使载物台徐徐上升至物镜离玻片约5mm处。用双眼注视镜筒内，同时按逆时针方向转动粗调焦螺旋使载物台下降，直到看到清晰的物像为止（注意不可在调焦时边观察边上升载物台，否则会使物镜和玻片触碰，压碎玻片，损伤物镜）。如一次看不到，应重新检查材料是否放在光轴线上，重新移正材料，再重复上述操作过程直至物像出现和清晰为止。

为了使物像更加清晰，此时可轻微转动细调焦螺旋使之最清晰。当细调焦螺旋向上或向下转不动时，即表明已达极限，切勿再硬拧，而应重新调节粗调焦螺旋，拉开物镜与标本间的距离，再反拧细调焦螺旋，约 10 圈左右，（一般可动范围为 20 圈）。有的显微镜可把微调基线拧到指示微调范围的二条白线之间，再重新调整焦点至物像清晰为止。

（3）低倍镜下的观察　焦点调好后，可根据需要，移动玻片使要观察的部分在最佳位置上。找到物像后，还要根据材料的厚薄、颜色、成像反差强弱是否合适等再调节，如视野太亮，可缩小虹彩光圈，反之则开大光圈。

4. 高倍镜的使用

（1）选好目标　因高倍镜只能将低倍镜视野中心的一部分加以放大，故在使用高倍镜前应在低倍镜中选好目标并移至视野的中央，转动物镜转换器，把低倍物镜移开，换上高倍物镜，并使之与镜筒成一直线（因高倍镜工作距离很短，操作时要小心，防止镜头碰击玻片）。

（2）调整焦点　在正常情况下，当高倍物镜转正之后，在视野中即可见模糊物像，只要稍调动细调焦螺旋，即可见到最清晰的物像。注意高倍镜下不得调节粗调焦螺旋。

初用一台显微镜时，要注意它的高、低倍物镜是否能如上述情形很好配合，如果高倍物镜离盖玻片较远看不到物像时，则需重新调整焦点；此时应从侧面注视物镜，并小心转动粗调焦螺旋使载物台慢慢上升到高倍镜头几乎要与切片接触时为止（小心勿压碎玻片标本和损坏镜头），然后再由目镜观察，同时转动粗调焦螺旋，稍微降低载物台至见到物像后，换调细调焦螺旋，使物像更加清晰为止。

（3）调节亮度　在使用高倍镜观察时，视野变小变暗，所以要重新调节视野的亮度，此时可以放大虹彩圈或用凹面镜。

5. 显微镜使用后的整理　观察结束后，应先降低载物台，取下玻片，切忌在高倍镜头下取、放玻片！转动物镜转换器使物镜镜头与通光孔错开再升高载物台，并将反光镜还原成与桌面垂直，擦净镜体，罩上防尘罩。仍用右手握住镜臂，左手平托镜体，按号放回镜柜中。

四、显微测量法

在生药的显微鉴定工作中，经常要用显微测量标尺测量所观察的微细物像的大小。测量长度的微量尺有载台量尺和目测量尺。

（1）载台量尺　为一种在载玻片中央刻有微细刻度的特制标尺。刻度全长 1mm，精确等分为 10 大格，100 小格，所以每小格 10μm。刻度外围有一小黑圈，以便易于找到标尺。载台量尺不作为直接测量物体长度使用。

（2）目镜量尺　放在目镜内的一种标尺，为一块直径 20 ~ 21mm 的圆形玻璃片，上面刻着精细刻度 50 ~ 200 个。目镜标尺是直接用来测量物体大小的。还有一种网格式标尺是用来计算数目和测量面积的。

（3）细胞及细胞后含物的测量　先将目镜量尺装入目镜的铁圈上，用载台量尺标化。首先转动目镜，移动载台量尺，使两尺的刻度平行，且一端重合，再找出另一端

的重合刻度，分别记录目镜量尺和载台量尺重合范围内的刻度，计算出目镜量尺每一小格在该物镜条件下的大小（μm）。例：用5×目镜和40×物镜，测得目镜量尺100格等于载台量尺的50格，即目镜量尺在这一组合中每格实际长度为5μm。测量细胞及细胞后含物时，被检物的长宽等于与之相当的目镜量尺小格数×5μm，即得。如果目镜改变时，必须重新标化和计算。

五、显微镜使用的注意事项

（1）应随时保持清洁。机械部分可用软毛巾擦拭；光学部分的灰尘必须用镜头刷拂去，或用吹风机吹去后，再用擦镜纸轻擦。切忌用手指或其他粗糙物如纱布等擦拭，以免损坏镜面。

（2）用显微镜观察时，必须同时双眼睁开，切忌紧闭一眼。要反复训练用左眼窥镜，右眼作图。

（3）用于观察的标本必须加盖盖玻片，制作带有试剂的玻片标本时，必须两面擦干后，再放在载物台上观察。

（4）如遇部件失灵、使用困难时，不可强行转动，更不可任意拆修，应立即报告指导教师解决，以免造成损坏。

Section 1 Structure of Microscope and Announcement of Usage

1. Types of microscopes

The microscope is an important and irreplaceable instrument in research of cytoarchitecture, tissue signature and organ construction, including main types as follows.

（1）Optical microscope It is a microscope whose lensis made of glass, using visible light as light source. They are classified as single and compound types. The compound microscope with complex structure is the commonest type in plants anatomy experiments and is made up of at least two groups of lenses, with the enlargement factor of 1250 and the highest resolving power of 0. 2mm. Besides the common biological microscopes for general microscopic experiments, there are other kinds for research, such as the darkfield microscope, contrast phase microscope and fluorescence microscope.

（2）Electron microscope It is the microscope using electron beam aslight source; its lenses are made of special electrode and magnetic pole instead of glass. It can distinguish any objects away from 2Å（1Å = 1/10000μm), and its enlargement factor can be 800 000 – 1 200 000 and resolving power is 1000 times of that of optical microscope. It is an important exquisite apparatus for ultrastructure.

2. The structure of compound microscope

The basic structure includes two parts: the optical system for imaging and the mechanical system for fixing optical system.

（1）Mechanical system

① Base A basic unit of microscope upholds the whole lens body and makes it steady.

② Column A short erect column on the base upholds the parts above it.

③ Arm An arm – likepart connects the base and the body tube for holding.

④ Body tube A long tube connecting the ocular and objective lens converter protects beam path and brightness of imaging.

⑤ Substage condenser A disc under the column can be turned freely. There are 3 – 4 screw poles for objective lenses on it. When it is turned, the objective lens can be fixed at acertain place to make the light of objective and ocular lens on the same line.

⑥ Stage It is a platform on which the specimen is placed. There is an opening in the center, through which light passes through. The clip on it can fix a slide and the turner under the platform can move the slide to every position. Sometimes there is a vernier on the stage.

⑦ Adjustments A deviceis used for bringing a specimen into focus. On both sides of the pole there are coarse adjustments and fine adjustments to move the stage up and down when it is turned. The bigger ones are coarse adjustments, which can move the stage about 2mm when they are turned a circle. The small couple is fine adjustments and the same operation makes the stage moved about 0. 1mm.

(2) Optical system This part is made up of imaging system and illuminating system. The imaging system contains an objective and ocular. The illuminating system contains a mirror and condenser adjustment. The microscope without mirror uses electric light.

①Objective lenses They are setted within the substage condenser and can be classified asthe lower – power, high – power, and oil – immersion lens. They magnify a specimen primarily. Enlargement factor and numberical aperture (NA) are wroten on them generally, for example, here is some data about XSP – 3C, the domestic product.

Multiple of objective lens	Numberical aperture (NA)	Working distance (mm)
4 ×	0. 1	37. 5
10 ×	0. 25	7. 31
40 ×	0. 65	0. 63

The working distance is defined as the distance between lens surface and a specimen. The smaller the working distance is, the greater enlargement factor will be. So should be more careful in use.

② Ocular They are assembled on top of the column and can magnify the image secondly. There are enlargement factors on them, such as 5 ×, 10 ×, 16 ×, and so on.

③ Mirror It is a two – side round mirror. One side is a plane mirror reflecting light; the other side is concave that can reflect and collect light. The turner on this mirror can turn over to various kinds of direction to reflect light on the condenser adjustment.

④ Condenser adjustment It is assembled under the stage and is made up of a condenser and an iris diaphragm. It can collect parallel light into a beam to make a specimen bright.

⑤ Iris diaphragm It is fixed in the condenser adjustment. We can adjust the size of the diaphragm by turning the operation bar as to control the light quantity.

3. Usage of microscope

(1) Taking and placing Take out a microscope from cupboard with the mounting number. You must hold the arm with your right hand and support the base with your left hand to make the whole body steady and erect. You shouldn't lift a microscope with a single hand; otherwise its screw will be loosed. A microscope should be put on the left side of your seat in a distance of 10 – 15cm from the table edge in order to observe and prevent from falling down.

(2) Adjustment of light We usually use the light shining from a window or the light from a daylight lamp but direct sunlight as a light source. Turn the low power lens to the center right onto the pore and observe a specimen with your eyes. Meanwhile, turn the mirror to the light source. You may use the concave mirror when light is weak. Once you see a bright round vision inside, turn the iris diaphragm to modulate light intensity to make the vision field clear.

(3) Usage of a low – power objective lens Whenever you observe a specimen, you should start with the low – power objective lens. The low – power objective lens can give a wider vision field so that you can find your goal and the objected part easily.

① Fix a slide Lower the stage, set a slide specimen right in the center of stage and make the point you want to the pore, and then fix the slide with two clips on the stage.

② Adjust the focus Look at the objective lens sideward, slowly turn the coarse adjustments clockwise to raise the stage until the distance between the objective lens and the slide is about 5mm. Observe the vision with your eyes and turn the coarse adjustments counterclockwise until you see the image precisely. (Don't raise the stage when observing, otherwise you may smash the objective lens and the slide). If you can't see any image, check whether your material is right on the light axis, remove it and follow the above mentioned points again till you can see your goal clearly.

In order to make the image clearer, turn the fine adjustment slightly. When the fine adjustment can't move up or down any more, it has reached its limit. Don't move it any more roughly, just readjust your coarse adjustment again, enlarge the distance between the objective and the specimen, and then turn your fine adjustment towards the other side about 10 loops (20 loops is a limit). In some microscopes the baseline of fine adjustment can be settled within the two white lines, and then the focus is adjusted to get a clear vision.

③ Test under a low – power objective lens After the focus is achieved, you can move the slide to the best place as you want. You could also adjust focus again according to the thickness, color of your material and the contrast degree of the image after you find it. For instance, if the vision is too light, turn down the iris diaphragm, otherwise, turn it up on the contrary.

(4) Usage of a high – power objective lens

① Search for your goal You should make your goal right in the center of the vision under the low – power objective lens before using the high – power one, because the latter only can

zoom in the central vision under the former one. Then turn the low – power lens away with a high – power one instead to make sure that it holds a line with the body tube. (Take care to prevent from breaking lens or slide because of the short working distance of a high – power lens)

②Adjust the focus As the high – power lens turns right, we can see the vision vaguely. After adjusting the fine adjustment a little, we can see it clearly. You must remember that coarse adjustment should not be turned under a high – power lens.

When you use a microscope for the first time, make sure if its low and high power lens match well as above. If not, readjust your focus as following. Observe the objective sideward, ascend the coarse adjustment slightly till it is going to touch the slice (Be sure not to break either a slice or lens), and then observe it from the ocular. Lower the stage by using coarse adjustment until the image appears, and then turn fine adjustment again to make the image clearer.

③ Adjust the brightness When you turn into a high – power lens, the field of vision will be smaller and darker. Readjust the brightness of your vision by magnifying the iris diaphragm or using the concave mirror.

(5) Tidying up After your experiment, lower the stage, get the slice off, turn the condenser to leave light condenser from the pore, lift the stage again, make the mirror be upright, make the whole body clean and cover the dustproof cover. Place the microscope back into the cupboard in the same way as you brought it out.

4. Microscopic measurement

When we work on microscopic identification of crude drugs, we usually use the micrometers included a stage micrometer and ocular micrometer to measure the size of minute image.

(1) Stage micrometer It is a special slide with a graduated meter at the center, which possesses 100 divisions of each 10μm in length of 1mm. There is a black circle surrounding the meter in order to easily find. The stage micrometer isn't used for measuring length of object directly.

(2) Ocular micrometer It is a meter on ocular, a disc slide with a diameter of 20 – 21mm and 50 – 200 fine divisions on it. Ocular micrometer is used for measuring length of object directly. Another kind is a grid like meter used for counting number and measure area of object.

(3) Measuring of cells and their ergastic substances Put an ocular micrometer onto the iron coil of ocular, and standardize it with a stage micrometer. Turn the ocular, move the stage micrometer, make the two graduations parallel when the one end of 2 micrometers are coincidence and find the coincident graduation of another end to note the graduation numbers of 2 micrometers in superposition scope. Calculate the length of each graduation on the ocular micrometer amount to that of the stage micrometer (μm). For example: for a microscope with 5 × ocular and 10 ×objective, the length of 100 graduations on the ocular micrometer equals to that of 50 graduations on the stage micrometer, so the length of each graduation on the ocular micrometer is 5μm. When cells or their ergastic substances are measured, their size is the data