B.Sc. III Year (Theory)

Semester –VI

Paper XX (C)

Microbiology and Disease Management

Unit-1

2. Microbial Techniques

A) MICROSCOPY

Dr. S. S. Patale
Associate Professor
Department of Botany
Smt. S. K. Gandhi Arts, Amolak Science and P.H. Gandhi Commerce College, Kada Dist. Beed
Email- sspatale@rediffmail.com Ph. 9823937501

2. MICROBIAL TECHNIQUE

A) MICROSCOPY

There were no suitable means of magnifying invisible organisms prior to the 17th century. Anton Van Leeuwenhock is honoured for providing the first accurate report on occurrence of bacteria with the help of his single lens microscope of simplest possible design. He could make lenses and using them to build magnifying glasses to provide a magnification of about 200 times.

Robert Hooke had used a compound microscope in 18th century, but these were incapable of good performance due to defects. During the 18th century these defects were gradually overcome by the following refinements:

1. Corrected eye piece and object lenses.
2. A condenser to focus light on the object
3. A thin glass cover slip to place over a liquid drop on a glass slide so that object within the liquid could be viewed in a flat plane
4. The oil-immersion lens to increase resolving power.

Later there took place many refinements in microscopy during the last century. At present different kinds of microscopes are available.

I) Simple or Dissecting Microscope:

It is a simple microscope consists of only one lens unit. This lens unit may even be an ordinary magnifying glass. Dissecting microscope is used either for dissecting the material or for less magnification i. e. only 5X, 10X or rarely 20X. It is mainly used for taxonomic studies, embryo separation etc.

A dissecting microscope consists of a basal foot and a limb. The stage made up of a simple glass plate, is attached to the limb. For light adjustment purposes a mirror is attached to the limb under the stage. Mirror can be moved vertically with the help of an adjustment screw at the tip of the limb is present a folded arm, on which a lens of definite magnification is fitted. Folded arm is moved to keep the lens in the desired position on the stage. The material to be viewed is placed on the stage. The eye is placed close to the lens. Folded arm is tilted to bring the lens over material. Light is adjusted by movement of the mirror. Focusing is done with the help of adjustment screw.

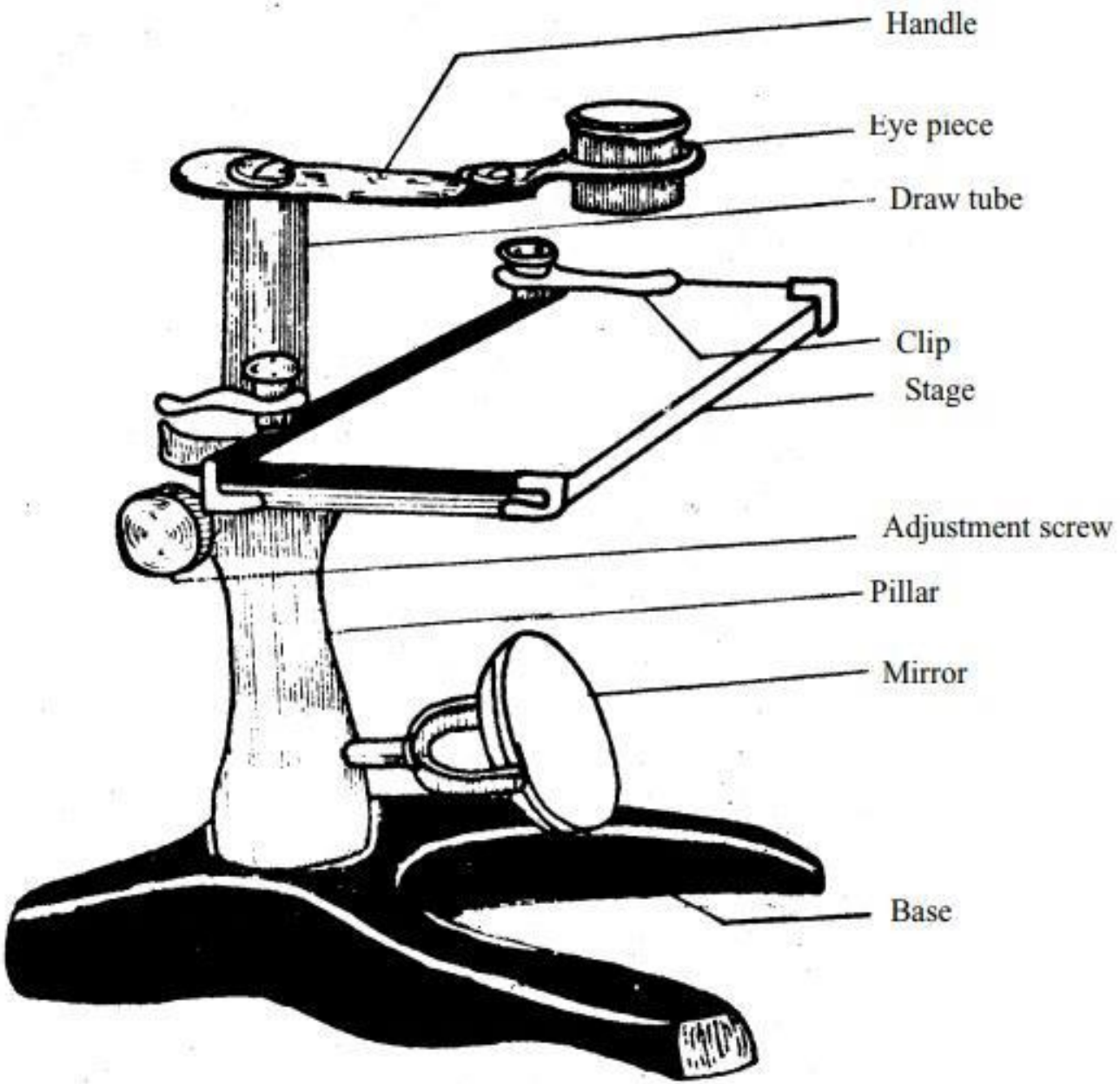
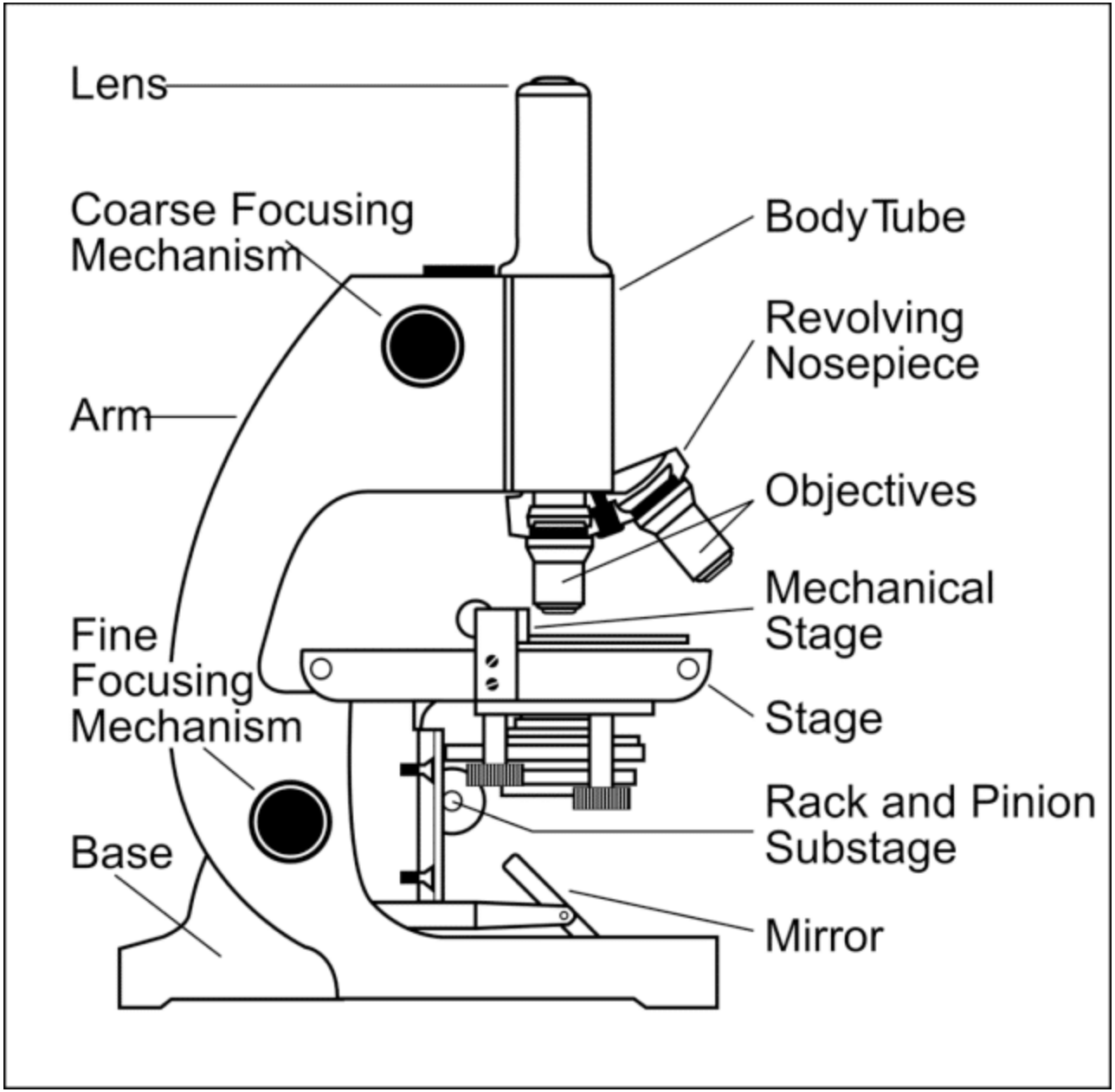


Fig. P.2A Simple Microscope

II) Compound Microscope:

It consists of two or more lens systems. At the top present the ocular lens. It can be turned around or may be removed. At the top of ocular lens is written 5X or 10X signifying the 5 times or 10 times magnification respectively. Just below the ocular is a body tube, the bottom end of which contains a circular piece, called nose piece. It contains three lenses called objective lenses. Nose piece can be rotated to change the position of objectives. The flat platform present below the objectives is called stage. On the arm of the microscope are present two knobs called coarse adjustment knob and fine adjustment knob. Out of the three objectives, the shortest is the low power objective. It has the largest lens but its magnifying power is least. On the objective may also be written 10X. It means if a 10X ocular lens is used the magnification is 10 X 10 =100. The other objective is high power objective.

Its magnification is equal to the number written on it multiplied by the power of ocular i.e. 5X or 10X. The third objective is called oil immersion. Generally it contains a black band around the lower end. Use a drop of oil on the slide at the time of studying with the oil immersion objective. Just below the stage is the condenser. Its function is to gather light from the mirror and direct it to the objective lens. Condenser may be lowered or raised by the knob present on the side of the microscope beneath the stage. Condenser contains a shutter called Iris diaphragm. Just below the condenser is present a mirror having its one surface flat and other con cave. Use the concave surface in the day light. Flat surface of the mirror is used when electric lamp is applied.


Lens
Coarse Focusing Mechanism
Body Tube
Revolving Nosepiece
Arm
Objectives
Mechanical Stage
Fine Focusing Mechanism
Stage
Rack and Pinion
Substage
Base
Mirror

III) Electron Microscopy:

The main point is the unusual short wavelength of the electron beams, substituted for light energy. The wavelength of about 0.005nm increases the resolving power of the instrument to fraction of nanometer. It makes possible to see viruses and large molecules clearly.

Two types of electron microscopes are in use today:

1) Transmission Electron Microscopy (TEM):

This is used to see the fine structure of cells. Ultra-thin sections of the objects are prepared by embedding or freezing the specimen and sectioning it with a diamond or glass knife. Sections are floated in water and picked up on a wire grid. They are stained with a heavy metal to make certain part dense, and inserted in the vacuum chamber. A 10000 volt electron beam is focused on the section and manipulated by magnetic lenses. A photograph prepared from the image may be enlarged with enough resolution to achieve a total magnification of over 20 million times. Objects as small as 1.0 nm may be observed.

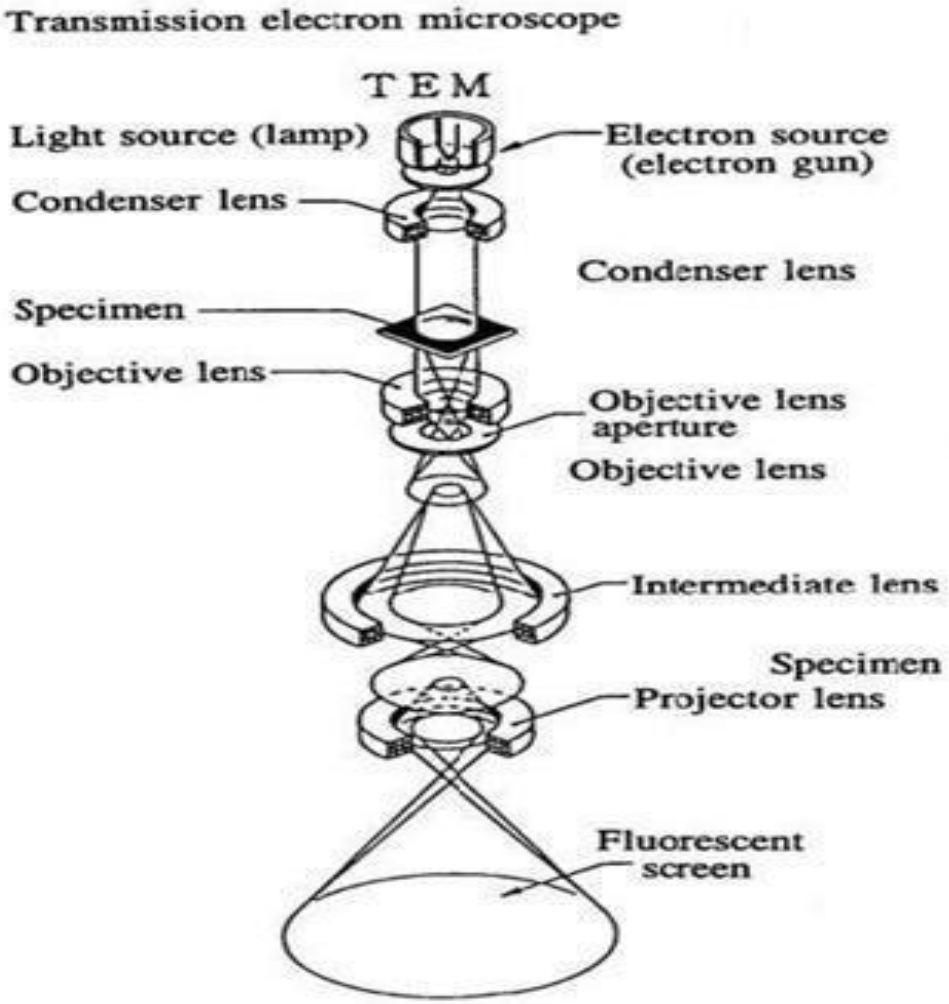

Transmission electron microscope
TEM
Light source (lamp)
Electron source (electron gun)
Condenser lens
Condenser lens
Specimen
Objective lens
Objective lens aperture
Objective lens
Intermediate lens
Specimen
Projector lens
Fluorescent screen

Fig 1

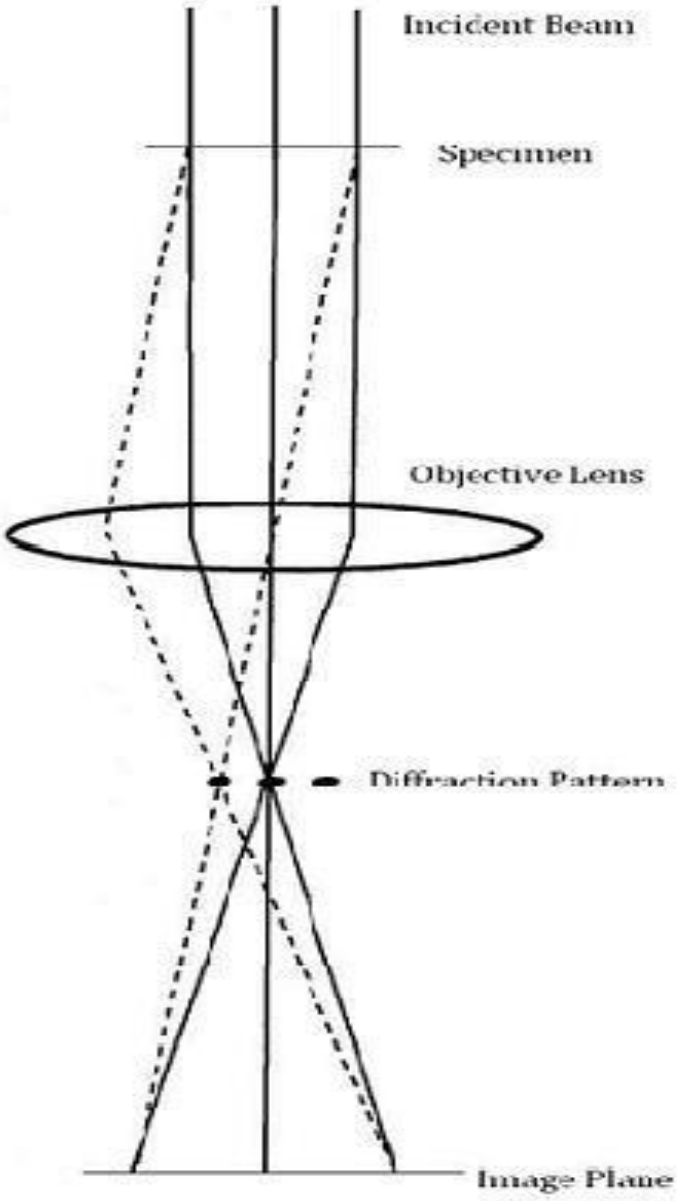
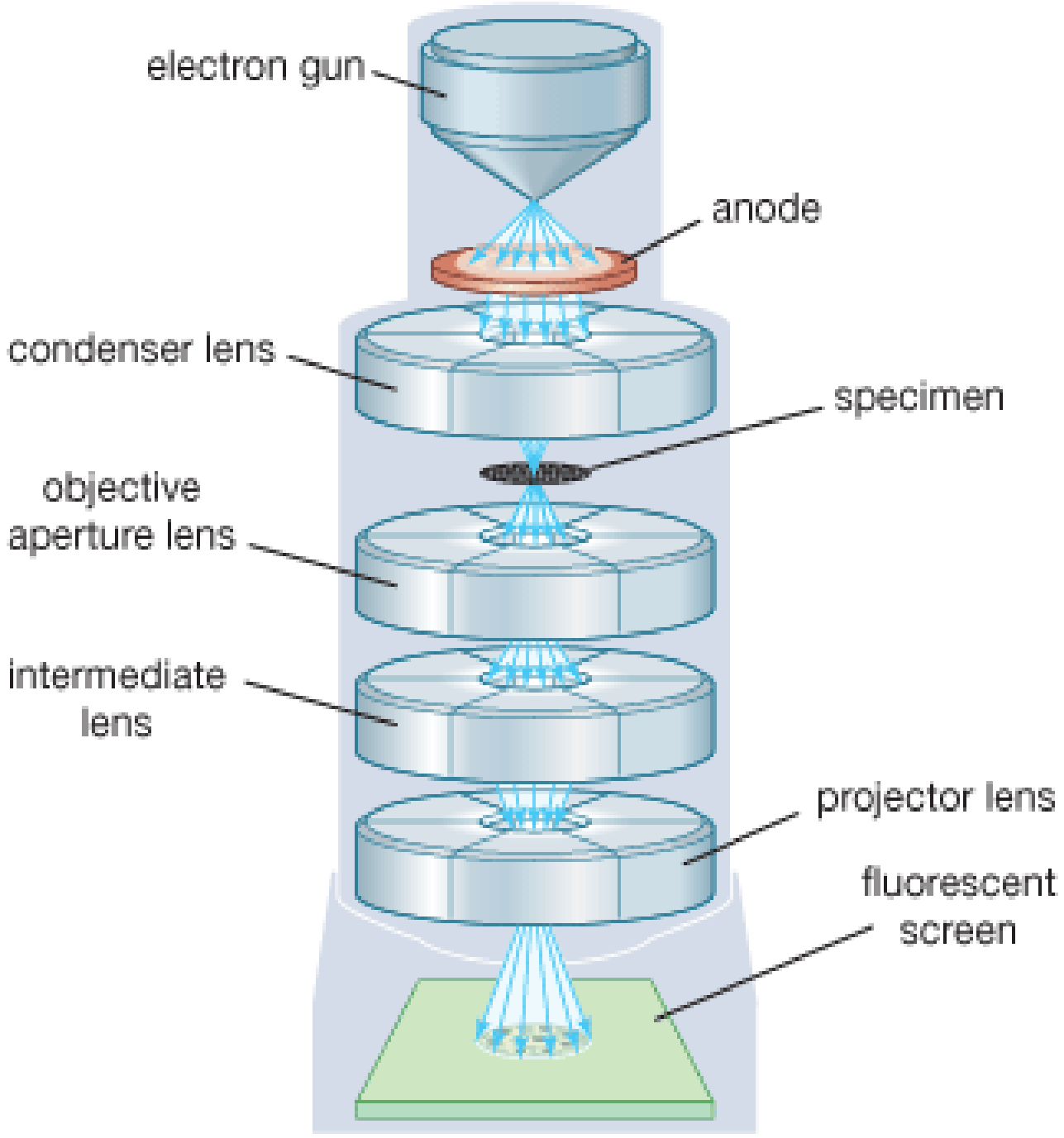

Incident Beam
Specimen
Objective Lens
Diffraction Pattern
Image Plane

Fig 2



2) Scanning Electron Microscopy (SEM):

This microscopy allows surfaces of objects to be seen in their natural state without staining. The specimen is put in to the vacuum chamber and covered with a thin coating of gold to increase electrical conductivity and thus forms a less blurred image. The electron beam then sweeps across the object building on image line by line as in a TV camera. As electrons strike the object they knock loose showers of electrons that are captured by a detector to form the image. Magnifications with this microscopy are limited to about 75000-100000 diameters.

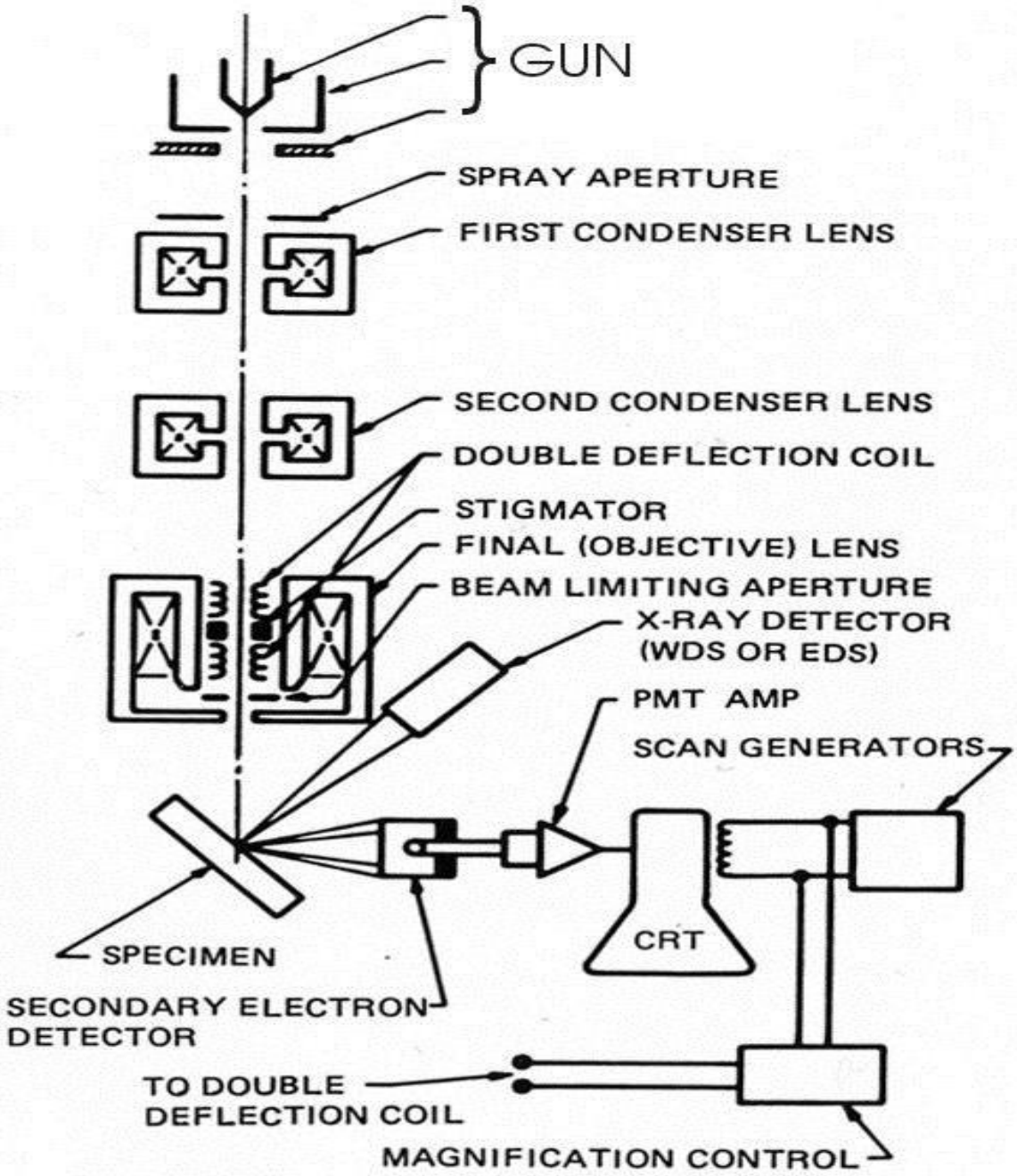


Figure 1.11. Schematic drawing of the electron and x-ray optics of a combined SEM–EPMA.


NOTICE
This equipment produces x-rays but is exempt from classification as a radiation-generating device.
Wave
X-Max
CAUTION
CAUTION
JEOL

Thank You