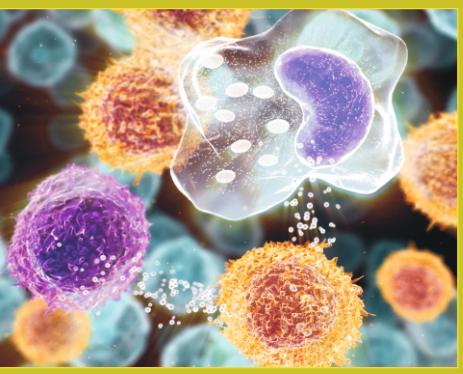
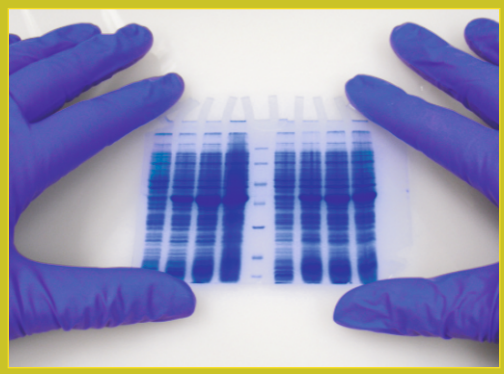
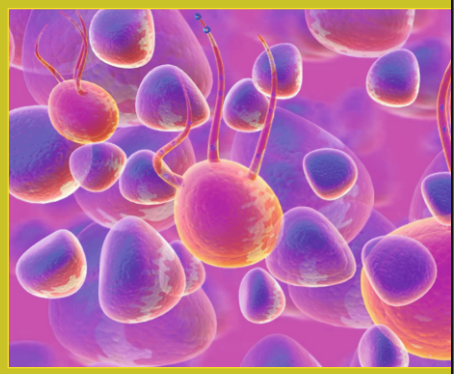
A BOOK OF

# BIOTECHNOLOGY

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# A Book of Biotechnology

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# CONTENTS

# PREFACE

The aim of this book is to present the subject of Biotechnology to the Graduate and Post graduate students of Biotechnology of various universities of India.

The authors have been extra cautious in presenting the matter in easy and lucid language. The diagrams are especially designed for clarity and simplicity. Thus, in this book many topics which are not easily available have been incorporated for the convenience of the students. So, the non-availability of literature, we started a sincere effort to search, collate, and edit the various topics. This was a challenging job. The completion of the book has not at all easy task. It has been completed with the co-operation of so many hands.

Suggestions for the development of this book shall be gratefully acknowledged.

— *Authors*

# ACKNOWLEDGEMENT

The authors wish to express their gratitude to Prof. Shakoor Khan, Principal Cambridge Girls P.G. College, Bhopal for his guidance and active participation in going through the manuscript.

Our thanks to other authors whose material has been put and utilized for promotion of the student knowledge of the subject through this book.

We further express our special thanks to our family members who were the source of inspiration in completing this academic effort.

— *Authors*

Chapter 1

# MICROSCOPY

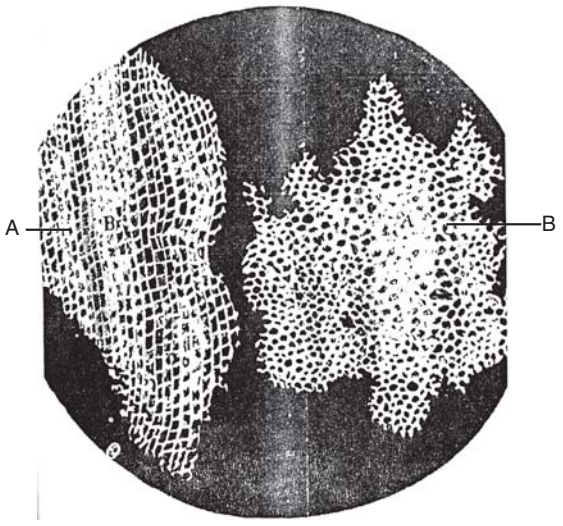
## INTRODUCTION

Almost all cells are too small to be examined directly with the human eye and so, our knowledge of cells has depended very much on microscopic techniques for magnifying them. The history of cell biology is an excellent example of the effect of one scientific discipline on another. The improvements in microscopy produced by developments in physics have been closely correlated with the expansion of cell biology. It is interesting to compare the appearance of the microscope used by Robert Hook in the seventeenth century with that of modern light and electron microscope. The magnifications attainable by these microscopes range from X 100 to X 400,000. In addition, several different kinds of microscopy are available, and many techniques have been developed by means of which specimens can be prepared for examination. Each type of microscopy and each method of preparing specimens for examination offers advantages for demonstration of specific morphological features.

## THE MICROSCOPE

Microscope is one of the most important instruments used in the biological sciences; its function being to produce an enlarged image of the object. For making "enlarged" images, simple lenses like "magnifiers" or "reading glasses" have been in use for centuries, especially in Ancient China. These are forms of simple microscope. Such simple magnifiers had definite limitations due to their crude construction. About 1590, a Dutch spectacle maker, Zacharias Janssen, used a second lens to magnify the image produced by a primary lens. This is the basic principle of the compound microscope used even today. Galileo invented an improved compound microscope in 1610.

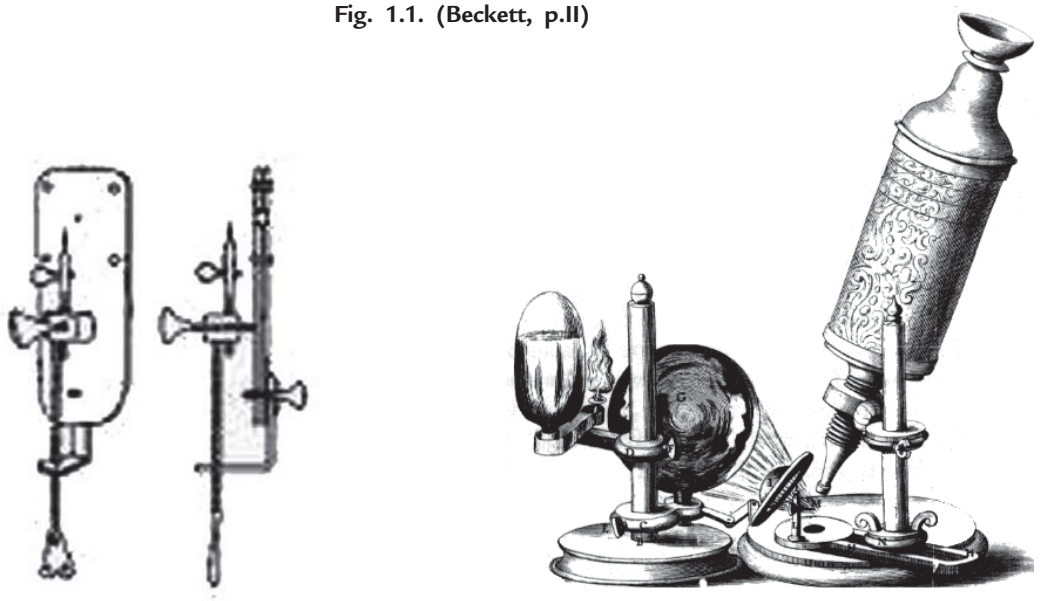
Antony Von Leeuwenhoek (1632-1723) was the first to record observations of bacteria, yeasts, and protozoa with the help of microscope invented by himself. A cloth maker and tailor by trade as well as the official winetaster of Delft, Holland, Leeuwenhoek's interest in




*Left:* In 1665 Robert Hooke made these drawing of thin slices cut from a piece of cork and viewed through his microscope. *A* is a cross-section, and *B* is a longitudinal section

*Right:* Robert Hooke's microscope

**Fig. 1.1. (Beckett, p.II)**



(A) (B)

**Fig. 1.2. Some early microscopes, used in the late seventeenth century :**

**(A) This microscope used by Leeuwenhoek consists of a single biconvex lens (I) inserted into a small hole on the left side of the leather base. The specimen was placed on the small pointed wire attached to the screw, which moved the specimen back and forth to bring it into the focus of the fixed glass.**

**(B) Hooke's compound microscope of the same period more closely resembles the appearance of a modern microscope. Note the elaborate decoration of the body of the microscope, the candle illumination source and the separation of the eyepiece from the objective lens.**

**(Atlas, p. 5)**

microscopes was probably related to the use of magnifying glasses by drapers to examine fabrics. Leeuwenhoek had the means and opportunity to pursue his hobby of lens grinding and microscope making. During his lifetime he made more than 250 microscopes consisting of home-ground lenses mounted in brass and silver.

The earliest microscope used by Leeuwenhoek was basically a very tiny, perfect lens that was used essentially like a magnifying glass. The difference between it and a conventional magnifying glass lies in the amount of magnification. A high-quality magnifying glass magnifies 10 to 20 fold, whereas Leeuwenhoek's best microscope magnified objects 200 fold. Leeuwenhoek mounted a specimen on the tip of an adjustable screw that he could position to focus the specimen. This is an example of a simple microscope. As early as 1847 Carl Zeiss Jena (Germany) built the first simple types of microscopes. The Olympus Optical Company was established in 1919 and began production of microscopes.

**Fig. 1.3. Antony van Leeuwenhoek (1632-1723) here seen holding one of his microscopes, opened the doors to the hidden world of microbes when he described bacteria. Although only a amateur scientist, Leeuwenhoek has a keen interest in optics, and his diligence allowed him to make this important discovery. (Atlas, p.5)**

## CARL ZEISS—A SYNONYM FOR QUALITY

The brandname Zeiss is a synonym for precision, perfection, and state-of-the-art design. This has been true of the company throughout its 130 years history. In the early days the small but exclusive Zeiss company in Jena had a standard answer to fastidious customers requesting products of special quality. "The Carl Zeiss Company guarantees identical high standards of quality for every product. No individual product is superior to the others. If this does happen to occur, then from that point on the entire production of that item is rigorously made to comply with those standards.

**Carl Zeiss (*1816–1888*)**

## NO COMPROMISE ON QUALITY

This guarantee was backed up by the name of the company owner: Carl Zeiss (1816-1888), a man who would countenance no compromises where his microscopes were concerned. If the slightest drop in quality was discovered in the products lining the shelves of his small shop, he would personally smash the entire batch with a hammer.

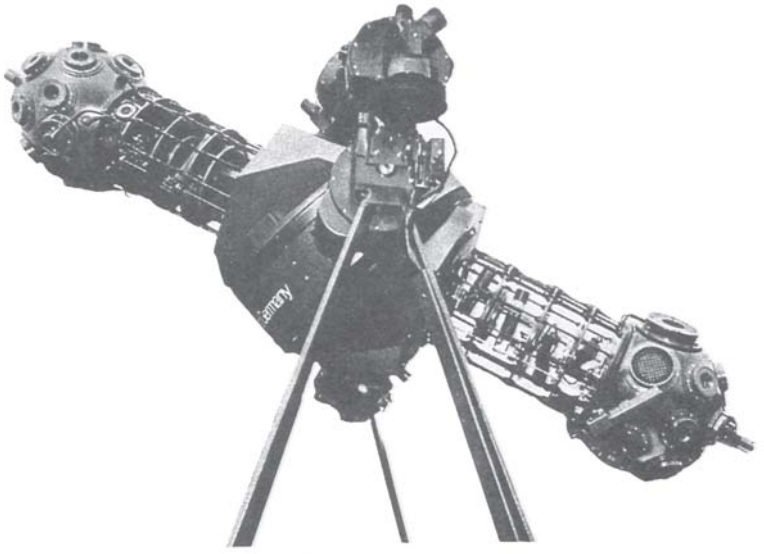


**Fig. 1.4. This large-scale planetarium projector, developed by Zeiss in Oberkochen, is a prime example of precision engineering.**
**(German News) Vol. XXVII, No. 3, March 21,1985**

Hard work and exacting standards were a family tradition. Carl Zeiss came from a long line of skilled craftsmen, and his father had been a master wood-turner.

His son had loftier ambition. At an early age Carl Zeiss developed an inquiring mind and later attended university lectures.

## RANKING AMONG PROBLEM-SOLVERS

Having becomes a qualified mechanic, Carl Zeiss joined the ranks of the problem-solvers. A hit- and -miss technique played a large part in the manufacture of his microscopes, and it was a time consuming process trying out hundreds of lenses until he had the right one. Often he had to rely on sheer good luck.

Carl Zeiss was not discouraged, however at the age of 50, he made the acquaintance of the 26-year-old Ernst Abbe, a private scholar from Jena. The two men complemented each other perfectly: the one a gifted but extremely sensitive physicist who suffered from recurrent bouts of migraine; the other an adventurous entrepreneur who never gave up.

It was nine years before the breakthrough finally came: the first comprehensive and exact scientific method of constructing systems of lenses.

## WORLDWIDE REPUTATION

By 1886, the Zeiss factory in Jena had already sold its 10,000th microscope. The company had long since established a worldwide reputation–not least thanks to the advent of a further expert in his field: Dr. Otto Schott, a specialist in the chemistry of glass.

After the Second World War the division of Germany also meant that the Carl Zeiss Foundation split into two parts. The special glass is today produced both by the state-owned company Carl Zeiss Jena in the GDR and by the Schott glassworks in Mainz. And the high-precision optical equipment bearing the brandname Zeiss is manufactured in Jena and in Oberkochen, a small town near Stuttgart, Federal Republic of Germany.

Before the many facets of microscopy are considered, it is important to understand a fundamental property of microscopes: *resolution* or *resolving power*.

## RESOLVING POWER

The problem of the magnification of an object is best considered in relation to the property known as *resolution*. The ability of lens system to show fine detail is termed its resolving power and this may be defined as the ability of an optical system to show as distinct and separate to points that are close together. Mere increase in size (greater magnification) without the ability to distinguish structural details (greater resolutions) is not beneficial.

The simplest illustration of resolving power is the visibility of double stars. Although the two stars may be separated by a vast distance, the visual angle reaching the eye is very small, and the stars appear to be close together. Many individuals can see but one star. Other persons, whose eyes have better resolving power can see the two stars distinctly. Applying this principle to the microscope, a lens of poor resolving power will show a slender chromosome as a single thread, whereas a lens of good resolving power will show the chromosome as two interwound threads.

The human eye has a fundamental limitation in that it cannot distinguish clearly points closer together than about 0.1 mm; it has a resolving power of about 0.1 μm. Points closer together than this will be seen as a single image. The resolving power of the eye is, in fact, less than that which is theoretically possible, due to the diffraction or scattering of light.

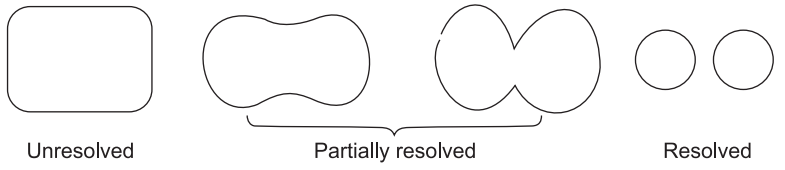



**Fig. 1.5. Resolution of two points under a microscope. At low resolution structures blur together; the greater the resolution, the more detail can be observed. (Atlas, p.43)**

Consider two points and the image that they produce at the retina of the eye. Ideally, in the absence of diffraction, these would produce two sharp peaks of light intensity. However, because diffraction occurs, a more diffuse area of light will be produced and the two images will overlap. The diffuse area of light is known as Airy disc and if the centres of the two

discs are closer together than the radius of each, the eye will not resolve the two points and will record only a single region of light.

The greater the resolving power, the greater the definition of an object, hence microscopes with high resolving power are especially good for viewing small structures. Resolving power in a compound microscope depends on the wavelength of light used and an optical property of the objective lens known as its numerical aperture. Since the wavelength of light is usually fixed, the resolution of an object is in practice a function of the numerical aperture; the larger the numerical aperture, the smaller the object resolved. There is a rough correspondence between the magnification of an objective lens and its numerical aperture; lenses with higher magnification usually have higher numerical apertures. (The value of the numerical aperture is printed on the side of the lens). But the medium through which the light passes also affects numerical aperture.

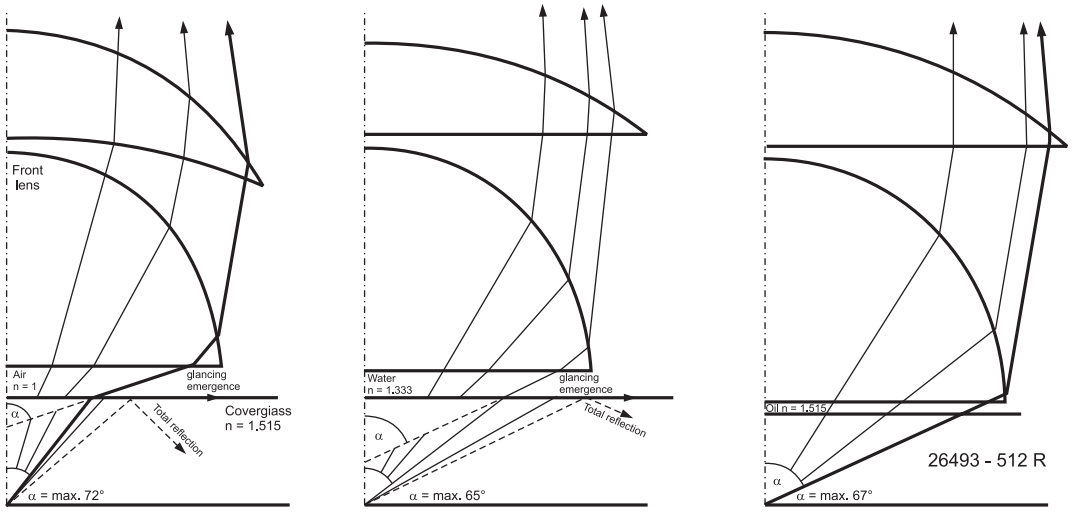



**Fig. 1.6. Diagrammatic representation of the aperture values of–**
**1. a dry system,**
**2. a water immersion objective, and**
**3. an oil immersion objective.**

The light microscope is able to increase the resolution of the eye because, essentially, it increases the aperture of the eye. This reduces the radius of the Airy discs and so allows the eye to distinguish points closer together.

By the end of the last century, great improvement had been made in light microscopy, and it was possible to obtain resolving power of about 0.2 μm, which is considered better than that of the unaided eye. However, the resolution of any microscope is fundamentally limited by the wavelength of the illumination employed. The average wavelength of visible light is about 550 nm, and so to improve on the resolution of a normal light microscope shorter wavelength illumination must be used. In some microscopes, ultra-violet light with a wavelength of about 250 nm has been utilized to give a resolving power required to see many cell structures. This problem has now been overcome with the development of the electron microscope which makes use of the wave-like properties of a beam of electrons. The electron

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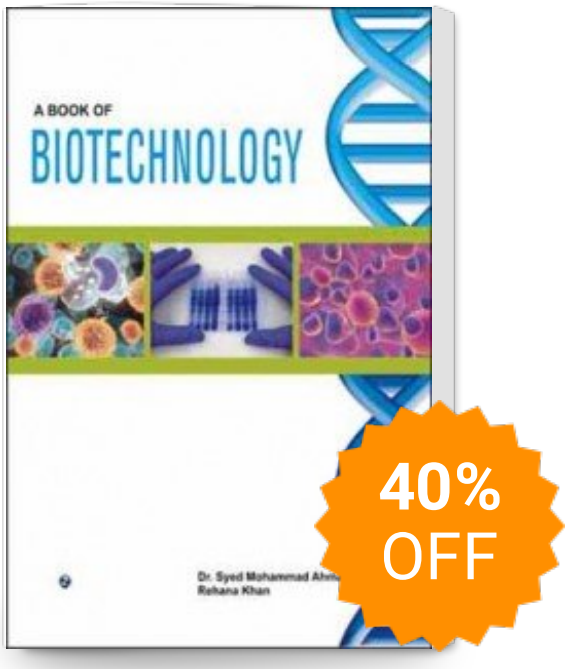


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