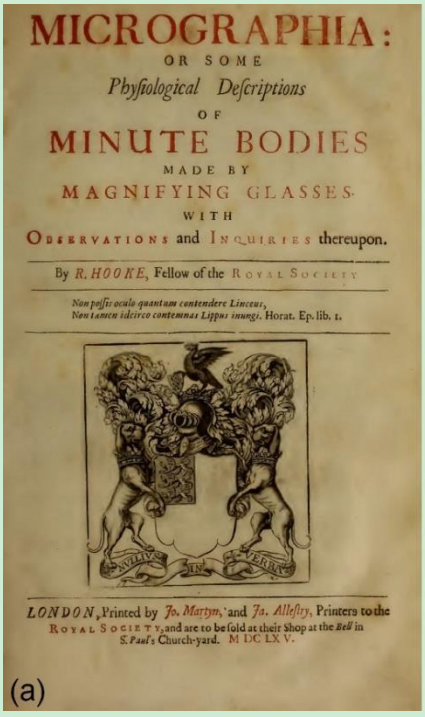
Bright-field Microscopy – A Short Introduction

Bright-field (or brightfield) illumination with a compound microscope is commonly used when observing specimens. If the contrast of a specimen is sufficient, bright-field illumination offers a combination of ease of use and performance. It is widely used for teaching microscopy at schools and for research applications in histology and histopathology. In this paper, we have to briefly discuss the basics of staining specimens for bright-field observation since features of very thin samples often require staining in order to be clearly visible with bright-field illumination.


Robert Hooke (1635 -1703)

Bright-field illumination (BF) is the fundamental technique for using a compound microscope. To set it up, establish critical or Köhler illumination, and the microscope is ready (see [**1**] for details about Köhler illumination). No other adjustments are required. Bright-field illumination is also the oldest technique used with compound microscopes. By the early 17th century, a variant called non-circular oblique illumination was already in use. This paper focuses on bright-field illumination, while oblique and darkfield techniques are covered elsewhere [**2**].

In 1665, **Robert Hooke** (1635 - 1703) published "Micrographia" (**Fig. 1a**), detailing his microscopy studies. This book includes his famous discovery that forms the basis of cell theory (**Fig. 1b**).


MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES.
WITH
OBSERVATIONS and INQUIRIES thereupon.
By *R. HOOKE*, Fellow of the ROYAL SOCIETY
Non possis oculo quantum contendere Lynceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.
LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LXV.
(a)

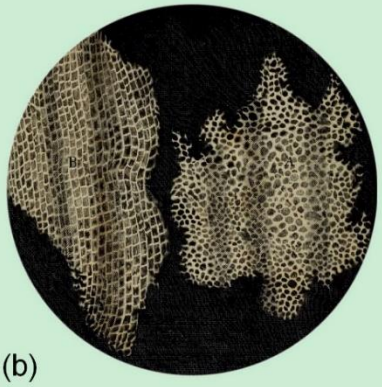

(b)

***Fig. 1**: (**a**) Micrographia: or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses. With Observations and Inquiries Thereupon, R. Hooke, The Royal Society, 1665. (**b**) The cut surface of cork as published in Robert Hooke's Micrographia. First illustration of plant cells observed with a compound microscope.*

In Micrographia, Hooke also discussed his compound microscope and presented details about the setup for illumination (see **Fig. 2**). The light of an oil lamp or wax candle was passed through a globe of brine that served to concentrate the light on a plano-convex lens [**3**]. This plano-convex lens could be moved around on an adjustable arm to focus the light. To protect the specimen from too much intense light, an oily paper was sometimes placed close to the specimen. With this setup, all specimens were observed in reflected light (not transmitted light). Hooke's microscope is also one of the first side-pillar compound microscopes [**4**]. Before this, tripod and drum designs were common. Although Hooke improved the compound microscope, he did not invent it. The invention is often credited to Zacharias and Hans Janssen [**5**], with earlier work by Francesco Stelluti (1577 - 1652) and Federigo Cesi (1585 - 1630) in 1625, and the term "microscope" coined by Giovanni Faber (1574-1629).

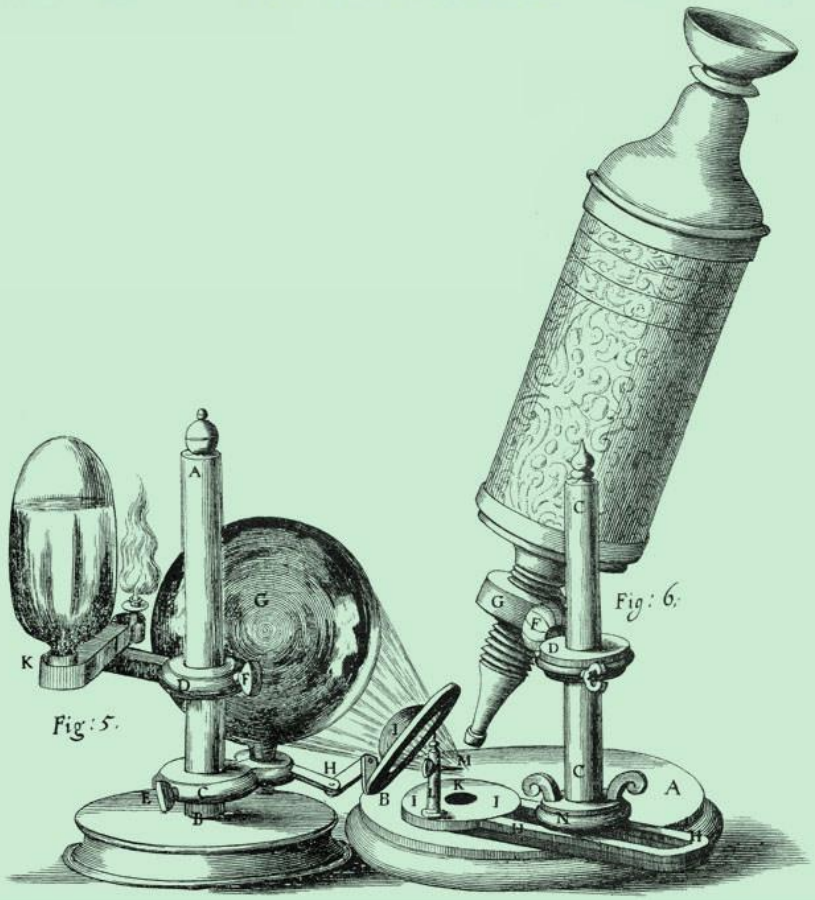


***Fig 2**: Robert Hooke's microscope, most likely manufactured by either Christopher White, Christopher Cock, or Richard Reeve following Hooke's instructions. This microscope was used for the observations leading to Hooke's famous account on microscopy "Micrographia" (published by The Royal Society in 1665).*

Bright-field illumination with transmitted light is most suitable for observing fixed, stained specimens or other types of specimens that naturally absorb a significant amount of visible light. Images produced with this illumination technique appear clearly against a bright background (often light gray or white). Below are several examples of photomicrographs using BF (see **Fig. 3**).

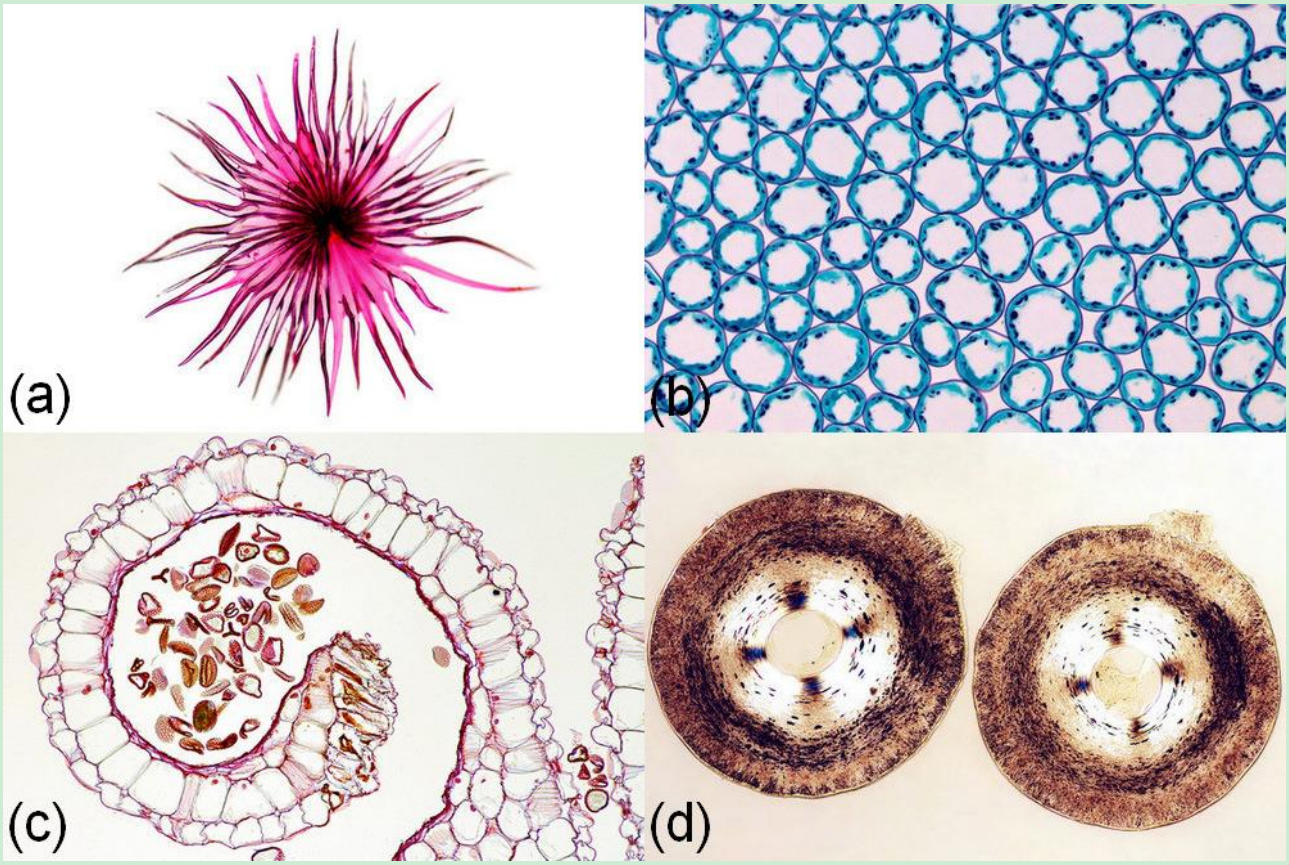


***Fig. 3**: Various specimen in bright-field illumination using transmitted light. (**a**) Hair of silverberry (Elaeagnus). (**b**) Leaf of lilac (Syringa); paradermal section. (**c**) Anthers of lily (Lilium); cross section. (**d**) Whiskers of lioness; cross section.*

Staining of Specimens for Bright-field Illumination

Successfully using bright-field illumination often requires careful staining of the specimen. In plant and mammalian histology, staining thin tissue sections involves a complex science with numerous chemicals (see [**6**] and [**7**] for a detailed account on this topic).

Fig. 4 shows the workflow for preparing histology slides with H&E staining at a sample thickness of 3 to 10 microns. **Fig. 5** and **Fig. 6** display examples of these stained tissue sections.

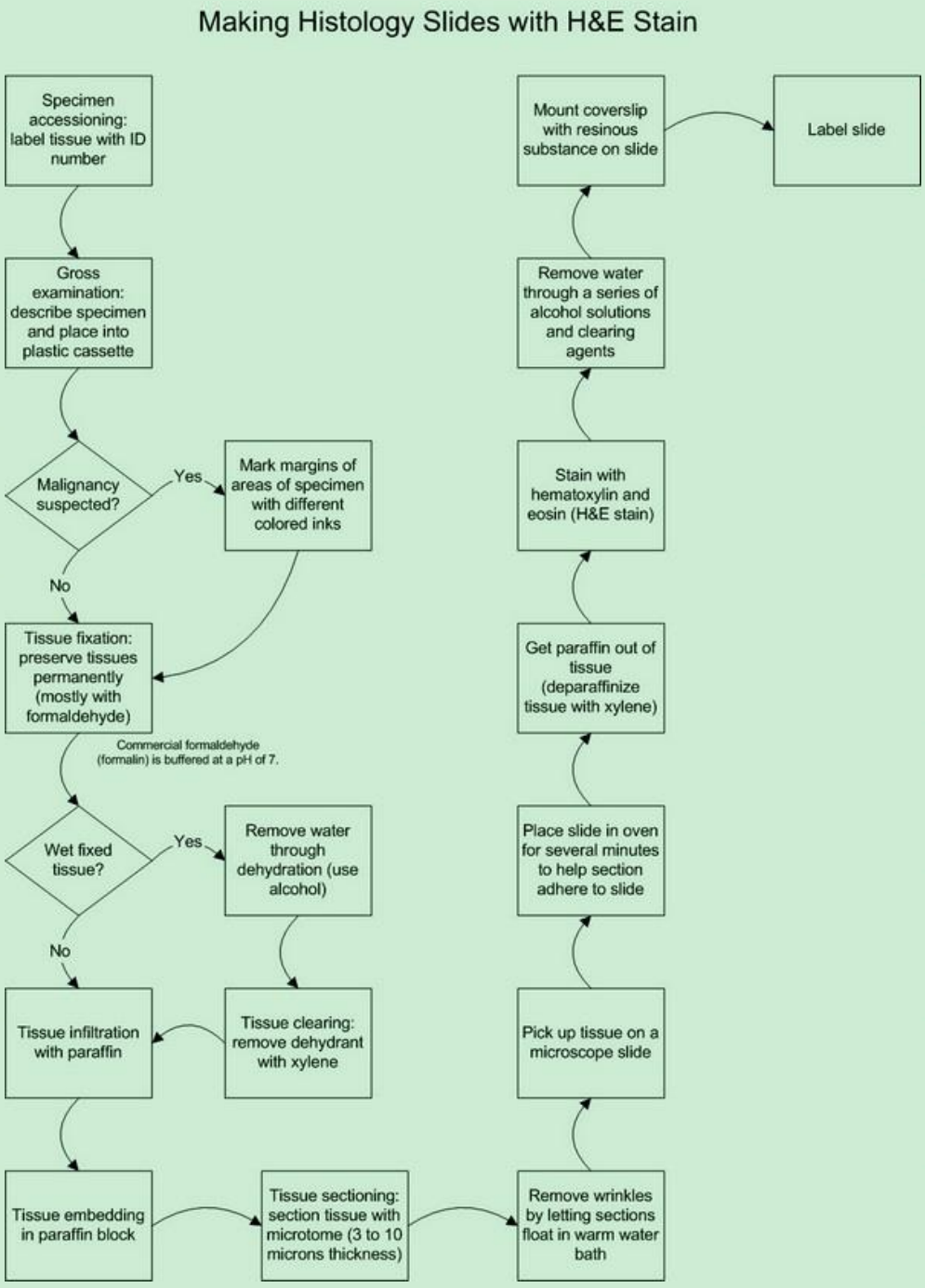


***Fig. 4**: Flowchart of standard techniques for processing tissues using H&E staining. At two points in this flowchart, a diamond-shaped box represents a decision with a 'yes/no' outcome.*

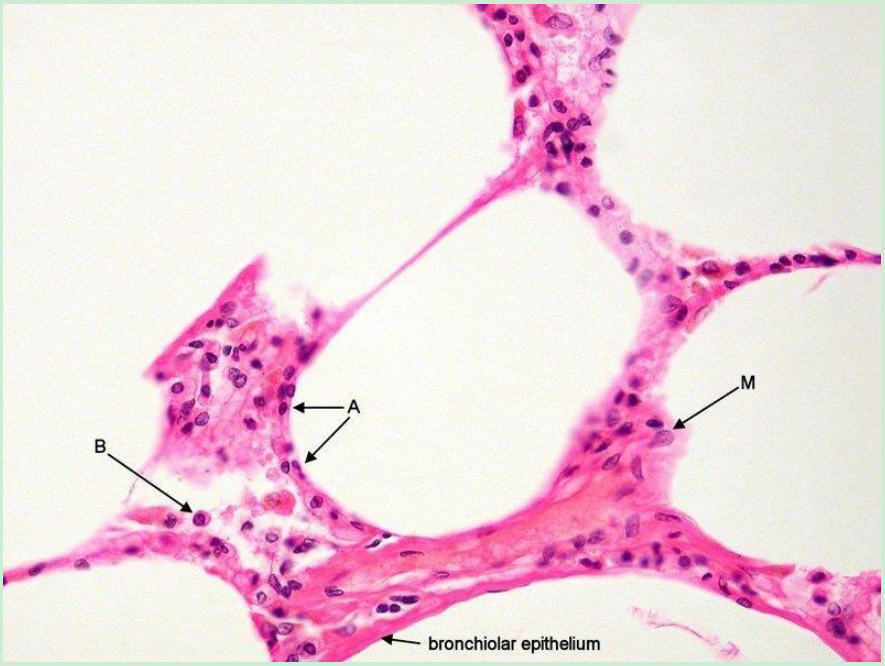


Fig. 5*: Section through human lung, showing the space of respiratory alveoli (objective magnification 40x). Shows two different types of epithelial cells. These cell types are called type I and type II pneumocytes and are indicated by the label 'A' and 'B', respectively. An alveolar macrophage can be found at location indicated by 'M'. Stained with H&E.*

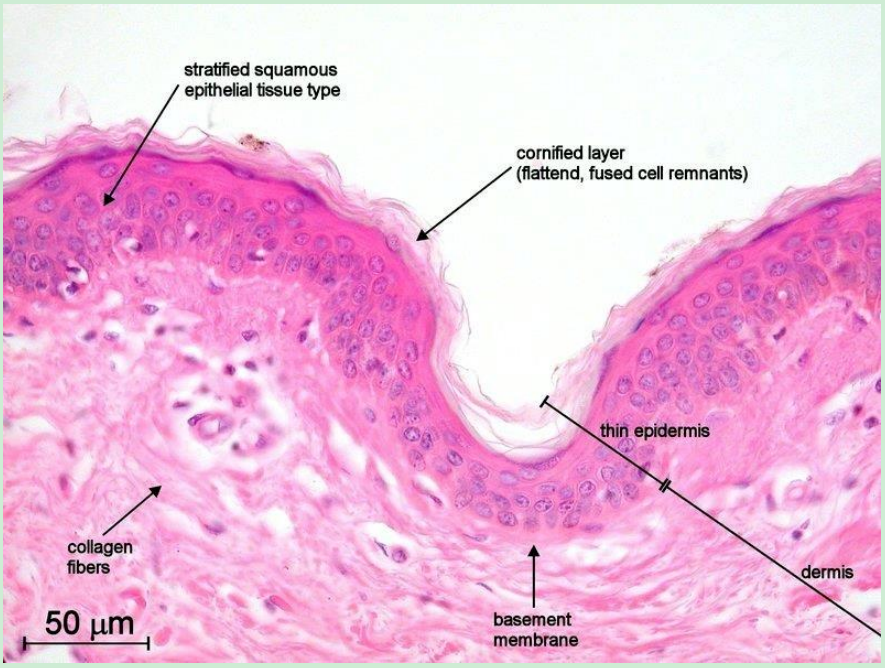


Fig. 6*: Section through nonpigmented, thin human skin (objective magnification 40x). Stained with H&E.*

Besides the very common H&E staining, there are many more complex staining techniques, such as Masson's trichrome staining and Golgi's heavy metal impregnation. Masson's trichrome technique developed by Claude L. Pierre Masson (1880 - 1959) highlights supporting tissues, especially collagen. **Fig. 7** shows a tangential section of a renal corpuscle from the cortical zone of a human kidney using this staining method.

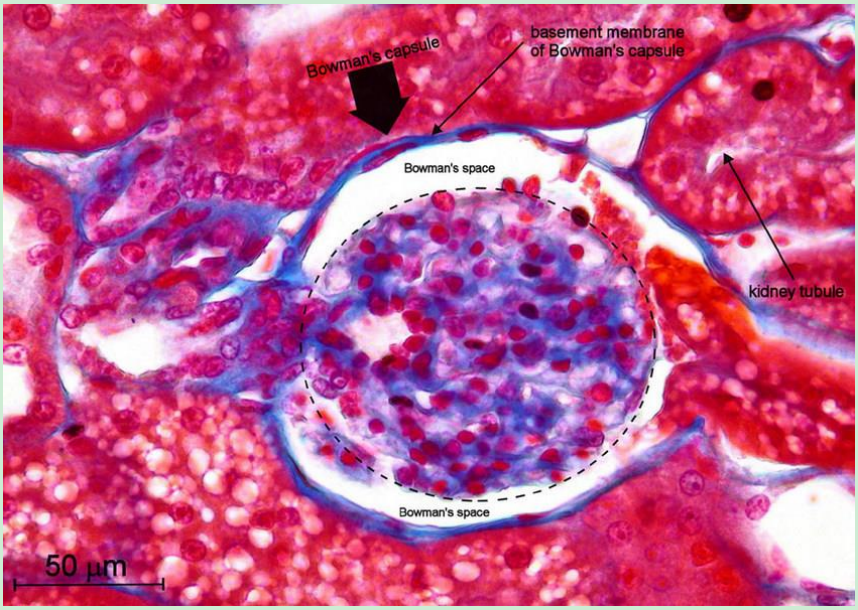


***Fig. 7**: Tangential section through renal corpuscles of cortical zone (objective 40x). The dashed line surrounds tuft of blood vessels (glomerulus). (Since this is a thicker section, the kidney tubules are not easily identified.)*

Finally, we highlight the heavy metal impregnation methods developed by Camillo Golgi (1843 - 1926), who created the first silver staining techniques for neural tissue. Ramón y Cajal (1852 - 1934) improved these methods. They allowed researchers to study the branching patterns of axons and dendrites in the nervous system. **Fig. 8** shows a Purkinje cell in the cerebellum stained with this technique.

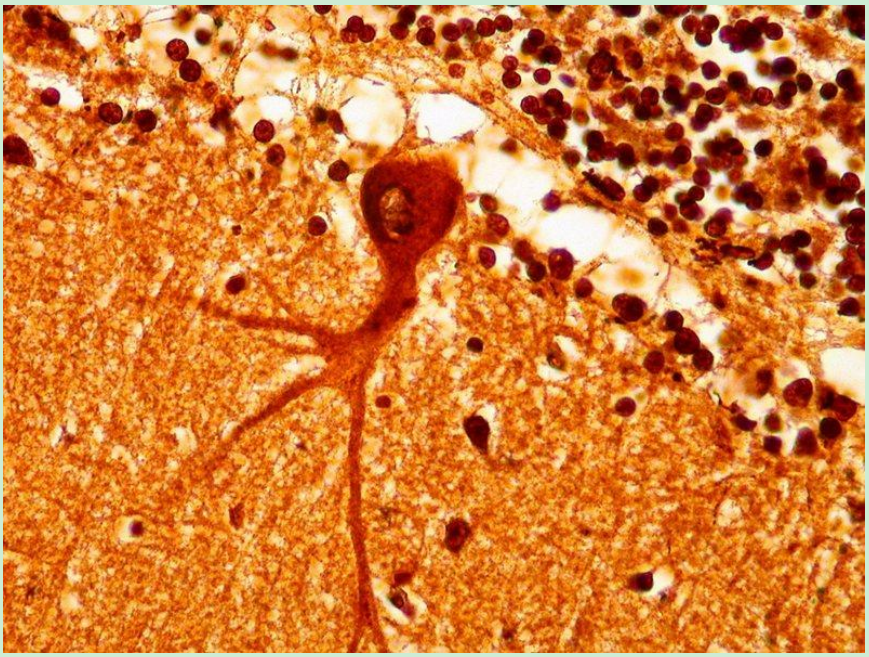


***Fig. 8**: Purkinje cell of the cerebellum using heavy metal impregnation staining.*

Instrumentation for Bright-field Microscopy

According to Bradbury and Bracegirdle [**8**], a compound microscope is an optical system where a real magnified image produced by one lens (or lens system) called the objective is further magnified by another lens called the eyepiece, resulting in a final, magnified, virtual image for observation by the user's eye. The authors note that while stereomicroscopes and macroscopes could also be classified as "compound microscopes" based on this definition, they are not typically included in this category due to historical reasons. A diagrammatic section through an early compound microscope is shown in **Fig. 9**.

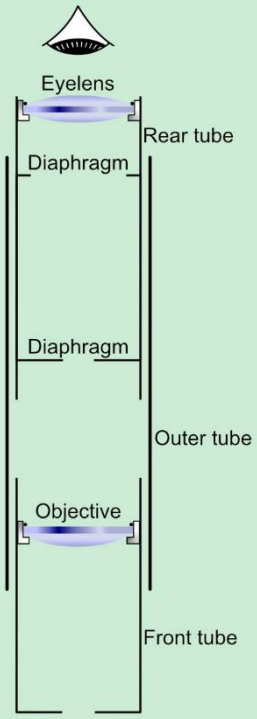


Fig. 9: *A diagrammatic section of an early compound microscope, similar to the design created by Janssen between 1590 and 1609. Each lens is mounted within a draw tube. This microscope was handheld and did not include a stand.*

A compound microscope consists of mechanical and optical components. Its performance relies on precise mechanical parts and high-quality lenses. **Fig. 10** shows the anatomy of a simple, older compound microscope. This type of microscope was frequently used in schools and at Universities in Germany.

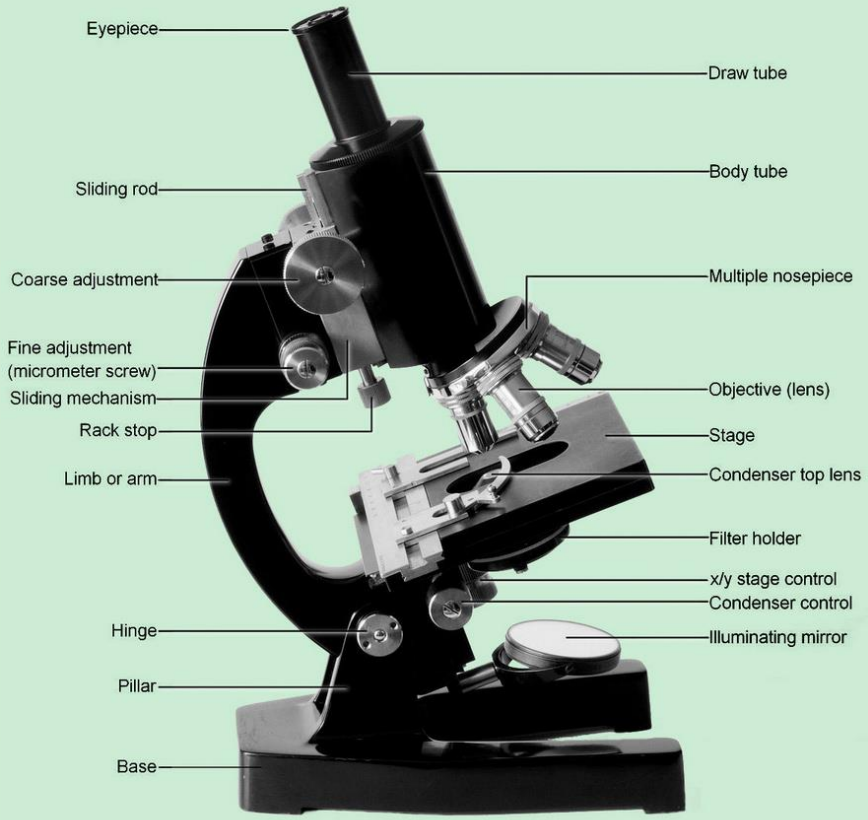


***Fig. 10**: The parts of a simple compound microscope.*

When light is generated by the lamp filament of a compound microscope using transmitted light illumination, it first passes through collector lenses and filters, followed by the condenser, microscope slide, specimen, cover glass (if present), objective lens, and ocular lens. Finally, the light reaches the observer's eye(s). Additional filters, prisms, and a tube lens may also be required to complete the optical configuration of a compound microscope. For example, in "infinity corrected" optical systems, a tube lens is positioned between the objective and the ocular lenses.

It is essential to understand that the resolution of a compound microscope depends on the relative positioning of all its optical elements, not merely the quality of each component. For instance, a microscope equipped with high-quality objectives and an excellent condenser will perform suboptimally if the front focal plane of the condenser (location of the contrast iris diaphragm) is not in focus with the back focal plane of the objective. In other words, the back focal plane of the objective and the front focal plane of the condenser must be conjugated aperture planes.

Fortunately, good compound microscopes suitable for bright-field work can already be purchased for a smaller amount of money. Buying a good, used microscope allows amateurs to access high quality systems at low cost. **Fig. 11** shows three basic types of compound microscopes for bright-field illumination.

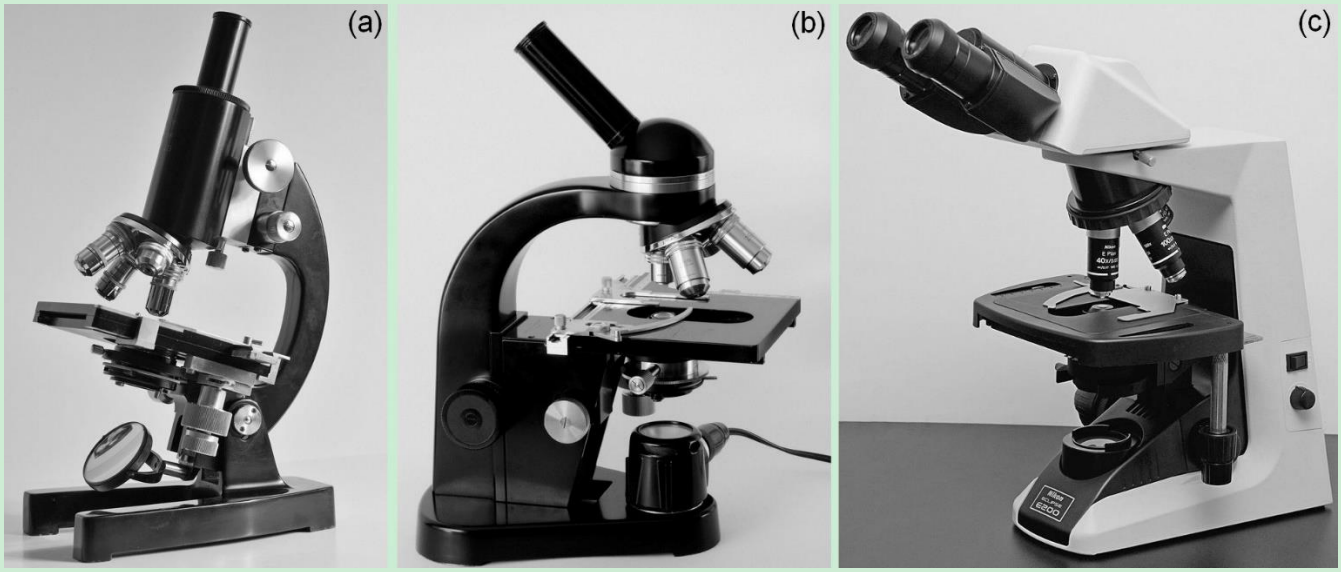


***Fig. 11**: Three basic versions of microscopes for bright-field work. (a) Basic microscope from Wolfe Wetzlar (less known manufacturer from Germany). (b) Better quality microscope from Leitz (Leitz SM). (c) A newer microscope from Nikon (Nikon Eclipse E200 Educational Microscope).*

The interested reader can find many more articles on using the compound microscope with bright-field illumination at http://www.microscopy-uk.org.uk/mag/indexmag.html. A nice paper published in Micscape Magazine is [**9**].

References

*[**1**] Gregor Overney, Köhler Illumination, Micscape Magazine, December 2017.*

*[**2**] Gregor Overney, Why I like Darkfield Illumination, Micscape Magazine, March 2004.*

*[**3**] S. Bradbury, The Microscope - Past and Present, Pergamon Press, Oxford, 1968.*

*[**4**] Gerard L'E. Turner, Collecting Microscopes, Mayflower Books, New York City, 1981.*

*[**5**] Between 1590 and 1609, the first compound microscopes were likely created. Hans Janssen, Zacharias Janssen, and Hans Lippershey, all spectacle makers from Middelburg in Holland, are credited for this invention. See [**3**] for more details.*

*[**6**] Peter Gray, The Microtomist's Formulary and Guide, The Blakiston Company Inc., New York, 1954.*

*[**7**] Stains File - A great site about staining techniques. Maintained by Bryan D. Llewellyn.*

*[**8**] S. Bradbury and B. Bracegirdle, Introduction to Light Microscopy, Bios Scientific Publishers, Springer-Verlag, New York (1998).*

*[**9**] Paul James, The Eye and Illumination with the Brightfield Microscope, Micscape Magazine, December 2005.*