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Microscopic Techniques for the Non-Expert

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Preface

In the era of nanotechnology, microscopic techniques are playing a vital role in any technological advancement of various multidisciplinary research area. Behind the scenes, nature always resides its secrets in a different form. One of the classical examples in the physical world is electromagnetic radiation (EM). We have known long before that EM radiation is like a wave, capable of showing interference and diffraction. The latter finding reveals one more additional property which is the particle nature of light composed of massless photons having discrete energy and momentum. This turns the two fold nature of light, the particle-wave duality. The light source and corresponding frequency levels are used to magnify the objects in the Microscope. The classical light source microscopes reported in the seventeenth century were able to increase the magnification by adjusting the depth of focus which could magnify as high as 1000 times of specimens. This could further lead to the origin of Microbiology field. The resolving powers of the light microscope are limited by 400 and 700 nm wavelength sources that are able to produce 200 manometers (0.2 microns, 0.2 μ meters). In the early 1930s, the introduction of the source of the electron beam as a magnifying source with the aid of a magnetic lens under the high vacuum resulted in the possibility of magnifying 100,000 times of specimens. The resolving power of electron microscope is capable of 0.1 nm is due to a few thousand of electron volt beam source. In 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, Stefan W. Hell, and William E. Moerner for having bypassed the limitation of optical microscopy resolution better than 0.2 mm. With the aid of fluorescent molecules, two separate techniques such as stimulated emission depletion (STED) microscopy and single-molecule microscopy are exploited to improve the resolution. Stimulated emission depletion (STED) microscopy—were used two laser beams, first stimulates to glow the fluorescent molecules, the second one selects the fluorescent with the nanometer-sized volume. While scanning the specimen by nanometer range, yields an image with a greater resolution which is better than the Abbe's stipulated limit. Single-molecule microscopy—this method helps to switch on individual fluorescence and scans the same area multiple times, each time scattered molecules glow. The final superimposed images, yields a dense super resolved image at the nano scale level. The implement of frequency modulation technique provides a high-resolution image even at the atomic level along with the chemical structure and surfaces of various samples. That is called scanning probe microscopy technique in the general category. It is based upon

scanning the probe on the surface of the samples, while it monitors some interaction between the probe and the surface. There are two state-of-the-art of interaction monitoring named as scanning tunnelling microscopy (STM) and atomic force microscopy (AFM). The STM can monitor the interaction between the metallic tip and a conducting sample surface to find the tunnelling current under the non-physical contact mode. AFM is capable of monitoring the van der Waals force between the tip and the surface. This could either function in contact mode (short range of repulsive force) or non-contact mode (long range of attractive force). Exploration of this technique is not only limited to the atomic scale resolution of visualization and manipulation of atoms but also able to study the biological important functional molecules (DNA, RNA, and proteins) interactions at the nanoscale domain. The notorious speech by Richard Feynman in 1959 at the California Institute of Technology (Caltech) accelerated toward the finding of the nanoscale dimension. Norio Taniguchi was the first to coin the term "Nanotechnology" ("Nano" means "dwarf" in Greece) in 1974. The progress of new microscopy technologies over the present technologies would lead to the expansion of new field of investigation associated with the technological development in the diverse field. This promotes invading of several technologically advanced processes in various disciplines like energy, environments, and health. Hence, it is mandatory for non-experts to know about the advancement of microscopy techniques. We hope this book would be helpful for us not only in the introductory level but also provide advancements in microscopy techniques to the non-experts.

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Sathish-Kumar Kamaraj is a research professor and Bio-Nano Interface Technology for Sustainable Energy and Environment research group leader at the Technological Institute of El Llano Aguascalientes, (ITEL)/National Technological Institute of Mexico (TecNM). He received his B.Sc. in Botany (Spln. Industrial Microbiology), Master's in Microbiology, and Postgraduate Diploma in Chemical Information Technology from Madurai Kamaraj University, Tamil Nadu, India. He obtained a Doctorate in Nanoscience and Nanotechnology (2010–2014) at the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV-IPN), CDMX, Mexico, with the scholarship from the General Directorate of International Relations-Secretary of Public Education Mexico (SEP-Mexico). During the academic period, he received the Best Student Competition Award at Battelle's Second International Symposium on Bioremediation and Sustainable Environmental Technologies (2013), in Jacksonville, Florida, USA. His thesis won the Best Ph.D. Thesis Award from the Mexican Hydrogen Society. His passion for sustainable natural systems triggers him to integrate his knowledge of various fields to address the problems in the area of Energy, Environment, and Health. He believes that the sustainable balance in the environment would lead to the wellness of the living being. He registered various patents in the Mexican Institute of Industrial Property (IMPI) and technology transfer to the industries. He also has relationships with the Government agencies and private sector for circumstantial decision-making. He served as a guest editor and reviewer in international journals.

Arun Thirumurugan is an assistant professor at the Universidad de Atacama, Chile working on the development of nanomaterial for energy and environmental applications. He has completed his Ph.D. (2010–2015) from the National Institute of Technology, Tiruchirappalli, India. He has worked as a postdoctoral fellow (2015–2017) at the Institute of Physics, Bhubaneswar, India, and then worked as a FONDECYT postdoctoral fellow (2017–2020) at the University of Chile, Santiago, Chile. He has vast experience in handling microscopes such as scanning, environmental, and transmission electron microscopes (FESEM, ESEM, TEM, and HRTEM). He has published 68 research articles and 9 book chapters in reputed

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A Beginner's Guide to Different Types of Microscopes

1

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Abstract

The microscope has been a great tool for the Scientifics since Zacharias Janssen was invented around 1600. The microscope is very helpful to study morphology and structural features (molecules to atomic) of the microorganisms (Virus, Bacteria, Fungi, and Algae), cell biology, and materials science. Over time, the microscope has evolved, with the aid of different sources of the electromagnetic spectrum that opens new avenues for visualizing seemingly invisible things from cell organelles to atomic resolution. Hence, it is vital to know about the different types of microscopy depending on the field of interest to use. In this chapter, we review the characteristics of the main microscopes. We focus on the types of emitting light sources, such as incandescent lamps, laser light, arc or flash lamps, and LED light. We also present the specifications of image (resolution, magnification, formation) as well as, the classification of microscopes (Compound, Electron and Scanning Probe Microscopes) and a comparison of various of their characteristics. In addition, we show a comparison of various characteristics of the microscopes. We review recent advances that leverage microscopy for robust image analysis.

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Keywords

Compound microscope · Emitting light sources · Image formation · Scanning probe microscope

1.1 Introduction to the Microscopic World

Microscopes were created to be able to see the smallest living beings and structures on the planet, far smaller than the human eye is capable of seeing unaided. The technology has, of course, developed much over time and today, the presence of modern optical instruments in laboratories dedicated to microbiological studies not only facilitates research but also allows us to obtain very precise results. Before using one of these powerful instruments, however, it is important to know a little about their functionality, as well as learn how to best use them. A microscope has three main functions: (i) to create a large image of the sample, (ii) to provide distinctly and, as far as possible, unequivocal information within the image obtained, and (iii) to make this information visible so that the human eye can comfortably see it, in order to make sense of it.

From its ancient origins with simple lenses over 4000 years ago to the invention of the first so-called compound microscope said to be by Dutch father-and-son team Hans and Zacharias Janssen in 1590, taking into account Galileo's discoveries on the way, the technology used to see the useable has developed with the desire to see evermore. Famously, Robert Hooke was the first to conduct experiments in which he magnified insects and sketched them, publishing Micrographia in 1665 [1] (Fig. 1.1). The microscope he used was lit by a kerosene lamp and used a spherical tank filled with water, a rudimentary microscope by today's standards and yet the images still a delight. Ever since these important, microscopic, milestones, scientists have not stopped observing the world through these, increasingly powerful, scientific instruments. Today, scientists can visualize not only the structures but also the dynamic processes within living cells and all of this in breathtaking detail. Modern microscopes are capable of revealing everything from insulin secretion to chemical crossfire in sections of biological tissue. However, modern microscopes still owe much to the compound microscope the Dutch spectacle makers, Janssen and Janssen developed all those years ago (Fig. 1.2); it consists simply of a pipe (tube) with a glass (lens) at each end. But how did the instrument work? Well, transforming the space between the glasses also modified the amplification. In the Fig. 1.1, we can see the basic concepts of the microscopic world seen in one of Hooke's compound microscopes.

1.1.1 Types of Emitting Light—Sources

Depending on the class of microscope being used, and the reason for using it, several options may present themselves when looking for a light source today. In the

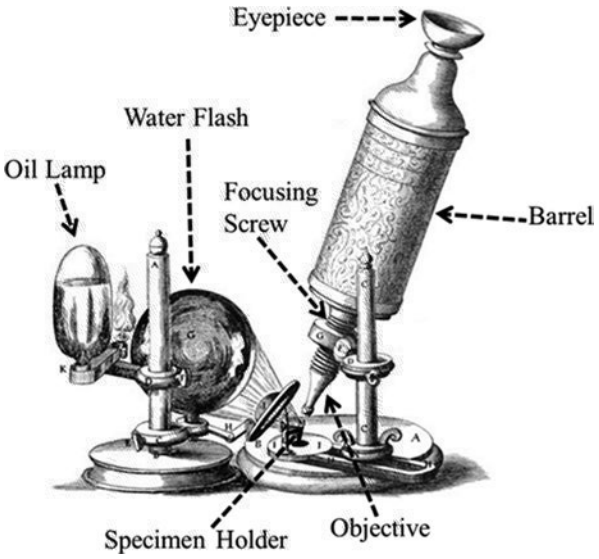

Fig. 1.1 Diagram of a compound microscope designed in 1660s by Robert Hooke. This image was taken and modified from Welcome Library, London; Copyrighted work available under Creative Commons Attribution licence CC BY 4.0: https://creativecommons.org/licenses/by/4.0/

past, ambient light from lamps and the sun were used to provide an external source of illumination, which were very effective in their day. Early microscopists commonly used ingenious methods of collecting and focussing light, using, for example, the reflection from a large whiteboard. However, a more reliable source of illumination was needed and thusly many alternatives have been developed over time.

The incandescent light bulb is still the most common light source for modern microscopes and is made of tungsten-halogen that is placed in a pensive casing, projecting illumination via the collecting lens and toward the subplate's condenser. To control the electric potential of the lamp, a resistor is used, which is incorporated into the microscope holder. The halogen tungsten lamp (bulb), consumes a constant current (CC) (voltage of 12 volts), which generates power of 100 volts. The DC power supply serves as a voltage controller for the lamp, commonly constructed into the microscope lodging, with a potentiometer controlled using an electric potential actuator button installed elsewhere on the microscope stand. Lightbulbs generally produce a great deal of heat throughout the microscope's operating period; however, the casing (made up of many layers of heat sinks) functions by dissipating the excess heat. Below, we present three types of emitting light sources.

1.1.1.1 Incandescent Lamps

As stated, these lamps are the main source of illumination used in modern microscopes; which are different from those used for investigations of fluorescence microscopy. These types of lamps transmit an uninterrupted spectrum of

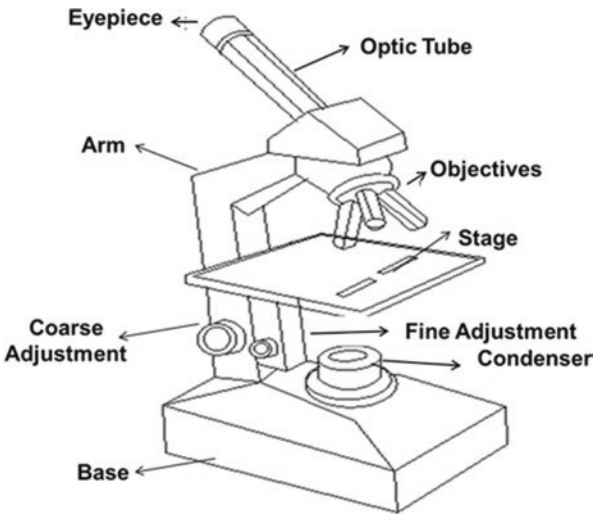

Fig. 1.2 Compound microscope design

illumination which ranges from 300 to more than 1200–1400 nanometers (nm). During the transmission of illumination's spectrum, most of the wavelength intensity is centralized between the ranges of 600 to 1200 nm area. Both the configurations, the construction and the operation of these lamps are very simple. They consist of a closed-lens light bulb stuffed with an inactive gas containing a wire filament made of tungsten fed with an electric current from direct current (DC). During operation, bulbs generate a lot of heat and light, yet this light represents only 5% of energy generation. The color, heat, and luminosity of these categories of lamps vary with the requested voltage, however, the average values are in a range between 2200°K and 3400°K [2].

1.1.1.2 Laser Light Sources

In the past few decades, the use of lasers, such as the argon-ion laser, which has a high emission capacity at 488 and 514 nm, has increased considerably. Lasers are very useful in laser scanning confocal microscopy, despite their high cost. The compact 405 nm violet lasers (VLs) were designed to replace expensive Ultraviolet lasers (UVLs) for most biological studies. The best performance of a laser is obtained when the maximum excitation wavelength of the dye is nearby to the wavelength of the laser. Other types of lasers are (i) Red Lasers (633 nm), and (ii) Green Lasers (543 nm), both consisting of helium-neon. In contrast, with these lasers, the maximum wavelength of the laser might not exactly match the maximum excitation of a given dye [3].

In addition, supercontinuum white light lasers are other categories of light sources that have been used in the past decades. In a comprehensive review on technology and applications of supercontinuum white light lasers, the author reported that those technologies could wide spectral coverage from 400 to 2400 nm [4].

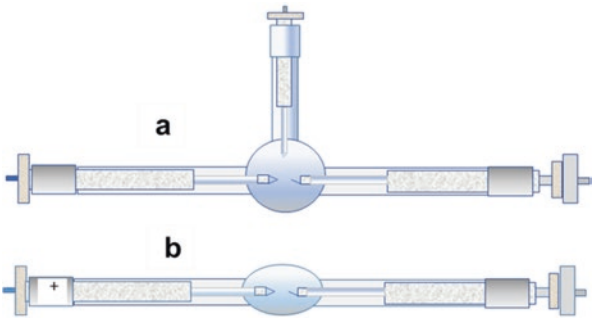

Fig. 1.3 Illustration of (**a**) a type of constructed lamp by the vapour of mercury (Hg) which is furnished with a lighter electrode, and (**b**) a contemporary small flash lamp (HBO 200-watt mercury) feeding with alternate energy via an outward power supply. This image was modified from Wikimedia Commons, copyrighted work available under Creative Common licence CC-BY-SA-4.0. https://commons.wikimedia.org/wiki/Category:CC-BY-SA-4.0

Supercontinuum white light lasers have great potential and the right characteristics to replace conventional light sources, due to their spatial beam profile, as well as their brightness and wavelength tuning combination.

1.1.1.3 Arc or Flash Lamps

According to Lagunas-Solar [5], flash lamps are also an important category in which mostly a gas discharge filled with xenon (Xe) is used. In order to function, an electric current must be employed to a compressed gas of up to 30 atm, allowing the passage of electric current and generating a brilliant wideband radiation emission, e.g., illumination. The spectrum emission of arc lamps includes (a) visible light, (b) alongside some infrared radiation, as well as (c) a significant band of radiation. Nitrogen (N) for vacuum UV can also be exploited to produce various spectra emissions to other UV bands. Arc lamps are built using fused quartz or glass tubes to avoid the absorption of so-called UV photons; Tungsten metal electrodes must also be used at both ends of these lamps.

In addition, mercury vapor-arc lamps (Fig. 1.3a) are another type of valuable light source for expert categories of microscopy [6]. Like arc lamps (Fig. 1.3b), they are also controlled by external lighting sources, which are designed to meet electrical requirements such as turning on the lamp first and then providing the proper current, while maintaining constant illumination. Flash lamps have an average life span of approximately 200 h, whereas most external energy sources are supplied with a timer allowing the microscopist to have control of the elapsed time.

1.1.1.4 LED Light Source

During the last decade, LED light sources have been applied frequently with the main objective of replacing traditional light sources, such as the traditional lamp. LEDs are promising emerging technologies used exclusively for illumination in light microscopy. These versatile semiconductor technologies possess all the desirable characteristics that traditional lamps lack. LEDs have been used because of

their high luminous efficiency, as well as their longer life span and excellent performance in terms of color. These light sources are widely used today. For example, if we want to work in different categories of fluorescent molecules, these are the best option as long as they are chosen with their appropriate spectrum at the time of their installation in the microscopes. Since its implementation, LEDs have been applied in Colibri lighting systems [7] and fluorescence microscopes [8]. Furthermore, LEDs have been used in the detection of individual molecules [9]. Some of the advantages that LEDs have are: their low cost, small size, improved the illumination of the images, provide a spectrum of multiple wavelengths; which are combined with a digital camera system [10]. Finally, LEDs are efficient enough to run on low voltage batteries, and this makes them very economical compared to other light sources.

1.1.2 Microscope Image

1.1.2.1 Image Resolution

The resolution of a person's sight is known to be 200 μm (0.2 mm), compared to a light microscope which has a high-definition capacity and can amplify images up to 1000× to illuminate details down to 0.2 μm [11]. The smallest distinguishable distance between objects (minimum resolvable distance) is known as the resolution limit. The resolution restriction of human sight is around 200 μm. However, the use of a light microscope allows the same items to be seen as two different entities, due to the light microscope's ability to easily distinguish distances of less than 200 μm. This indicates that there is a proportional relationship between the minimum resolvable distance and the resolution of an optical microscope, i.e., the shorter the distance value is, the larger the resolution of the microscope [12]. In Fig. 1.4, we present the different parts of a Light Microscopy.

1.1.2.2 Image Magnification

Theoretically, image enlargement is the process by which the resolution of the image is virtually increased in sequence to highlight details that are implicit in the original image, but not obvious [13]. Image enlargement has different applications. These include, (i) the analysis of satellite images [14], (ii) the visualization of medical images, and (iii) the coincidence of images captured with different sensors. Generally, this type of image requires a large amount of memory (high capacity) to be constituted employing a bidimensional matrix of pixel worth. When using various encoding schemes, the memory demand for warehousing for transmitting is reduced. Furthermore, the bandwidth requirement depends on both (a) the dimension of the image and (b) the type of procedure utilized for encoding. Ideally, it will continue expanding the image by improving the magnification [11]. However, it is difficult to reveal new information of consistent quality on a piece (object) just by enhancing the magnification. For this reason, before applying the technical magnification to any encoded image, it must be transformed to regular form across the decoding procedure, which incurs a few computer-based costs. Additionally, clear

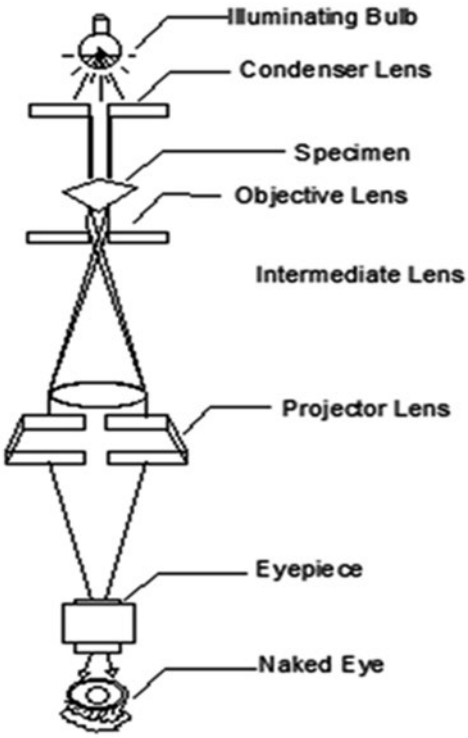

Fig. 1.4 Shematic diagram of a Light Microscopy

Table 1.1 Dimension of the image components through different amplifications for 2000 × 2000-pixel picture or image settlement and 20 × 20 cm exhibit magnitude

Magnitude of image component on the sample	Magnification
10×	10×
1.0 μm	100×
100.0 nm	1000×
10.0 nm	10,000×
2.0 nm	50,000×
1.0 nm	100,000×
0.10 nm	1,000,000×

Note: This table was modified from Ul-Hamid [11] with permission of Springer Nature (Licence number: 5201530877446)

information in an image cannot be determined beyond a certain amplification (magnification); because of the restrictions imposed by both the solving energy of the imaging technique and those of human sight. According to UI-Hamid [11], the magnitude of the image component can be heightened by using a smaller size. Table 1.1 shows the magnification during imaging.

1.1.2.3 Image Formation

The illumination from the optical microscope flashlight moves, by way of the condenser, throughout the sample (specimen), some of the illuminations cross around and some are received via the sample, having been calmed on its way. These types of illuminations are known as deflected, or direct illumination (light). During the image formation, the electron pipe located in the highest area of the column produces an electron beam with power in the range of between approximately 100 eV to 30,000 eV. This is concentrated on a fine probe through so-called electromagnetic glasses (lenses), found inside the column.

In addition, the light diffracted by the sample is focused in different places located on a similar image plane. This diffracted light then causes harmful interference, reducing the power and outcome even in less obscure regions. During the imaging process, the electron ray penetrates the specimen in a teardrop form, expanding with values from100 nm to 5 μm which depend not only on the density of the sample but also on the energy of the beam. Another aspect to consider in this process is the fact that there is an interaction between both the beam and the specimen, producing several indicators including electrons and backscattered X-rays [11, 15]. These are self-possessed and utilized to determine the basic creation of the material sample, as well as producing the images. Figure 1.5 indicates the connection established, one by one, between the points on the display screen and the impact points of the beam on the sample surface.

Furthermore, electrons expanding from a separate position are identified with the support of a kind of detector as an indicator with a determined intensity. This way, from each end (point), where the electron ray relates to the sample and also generates an indicator (signal), it connects the corresponding end on the display monitor; it is displayed as an intensity (I).

Finally, the type of lens is a key factor in image formation. Modern microscope lenses are capable of obtaining real-time images of the samples with the minimum

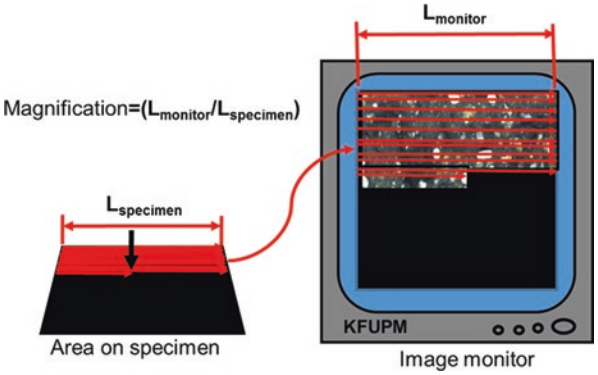

Fig. 1.5 Illustration of the one-to-one connection between the beam locations on the points on the monitor and the specimen (Modified from UI-Hamid [11] with permission of Springer Nature; Licence number: 5201530877446)

possible margin of error. There are different types of microscopes and lenses that are used by researchers in laboratories today. These include (i) the objective lens which helps in the magnification strength needed, (ii) the eyepiece lens that researchers use to view the samples on the slide, (iii) the condenser lens that focuses the light source from the microscope onto the sample (condenser lens can obtain 400× magnification), and (iv) oil-immersion lens which is different with respect to the other lenses. This difference is because you have immersion oil between the lens and the glass slide. In addition, light microscopes are made up of two ocular lenses (5× and 10×) and three objective lenses (5×/10×, 40×, and 90/100×) [16]. Unlike light microscopes, electron microscopes use objective lenses, as well as projector lenses, convergence lenses, intermediate lenses, and eyepieces. Therefore, we can argue that the type of lens affects both the design and the operation of the microscope. The efficiency of a microscope is proportional to the type of lens used.

1.2 Classification of Microscope

Compound microscopes are manufactured to supply an expanded (magnified) bidimensional image, which can be centered radially on consecutive focal planes, thereby, allowing a thorough scan of a specimen's fine structural formation in both 2D and 3D [17]. Various categories of microscopes have been designed, configured, developed, and applied throughout numerous fields of science and technology (Fig. 1.6); however, the vast majority of microscopes could be classified into three

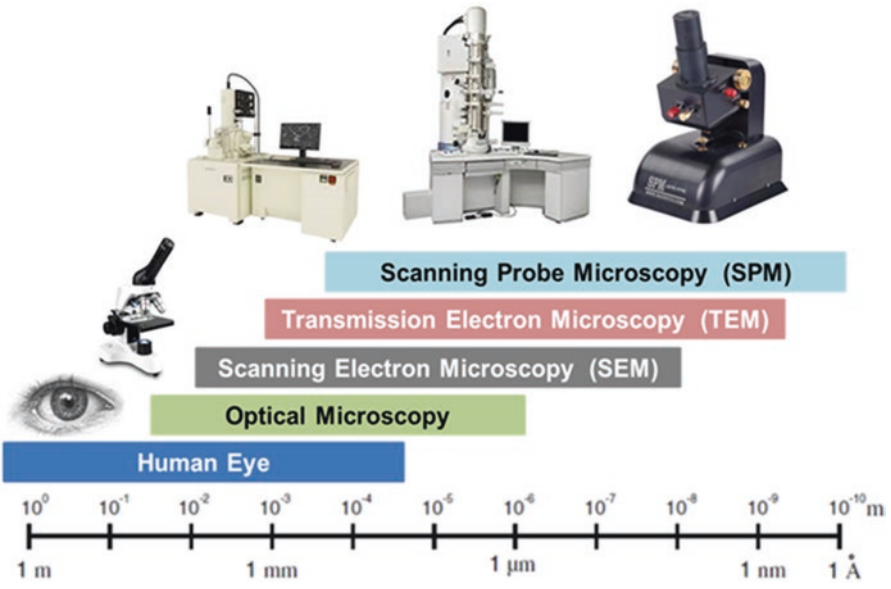

Fig. 1.6 Range of images for different microscopes

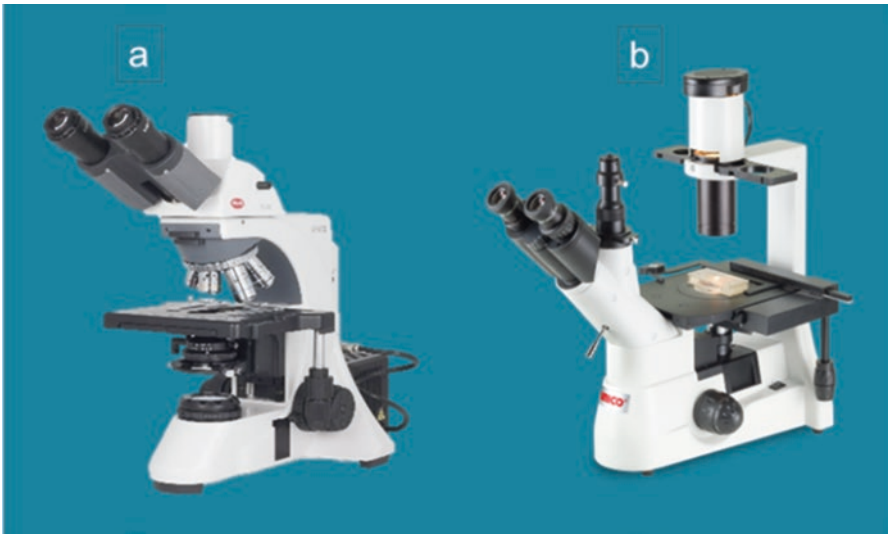

Fig. 1.7 Upright microscope (**a**) and inverted microscope (**b**)

classes: (1) electron microscopes, (2) fluorescence microscopes, and (3) optical microscopes [18].

The human eye can discern objects down to approximately 0.2 mm. Optical microscopes reveal minute objects that could not be seen in any other way and all by using the age-old method of enlarging them with the aid of a combination of glass lenses.

Microscopes can also be classified based on their construction. An upright microscope (Fig. 1.7a) that observes a specimen from above is by far the most well-known and has a seemingly endless number of applications. A reversed microscope (Fig. 1.7b), on the other hand, with its illumination source and capacitor on the top, observes a specimen from below.

An electron microscope with an enlarging lens processes an image of the object from above. Specimen magnifying glasses, however, are biconvex, meaning they are denser at the center than at the edge. The image is seen by the eye as if it was at 25 cm.

1.2.1 Optical Microscope (Light Microscope)

The optical microscope creates a magnified image of a specimen and serves three basic functions, (a) modifying an amplification (magnification), (b) sharpening an image, (c) bringing it into focus, and (d) obtaining clarity. This is also known as an Observation Optical System. The function of illuminating a sample consists of three fundamental roles (i) modifying illumination power, (ii) collecting illumination, and (iii) supplying illumination. It is also known as a Light Optical System. In

addition, a specimen can be projected throughout an optical system [19] and result in a protrusion image to the eyes or a pick-up apparatus such as a CCD (charge-coupled device). Nevertheless, the brightness of the said optical system, is in fact, a result of accumulated illumination emitted from the illumination source and gives the subject the brightness needed to enlighten it. Furthermore, for an inverted microscope, the arrangement liaison between those types of optical systems is, of course, reversed at the center of a sample as compared to an upright microscope. In a system of optical microscopy, monitoring at high heat is accomplished employing a UV CCD camera and UV light while radiant heat is cut off by a UV illumination transference filter [20].

1.2.2 Fluorescence Microscope

This category makes use of a very elevated intensity illumination source that moves a fluorescent source over a useful specimen. These fluorescent sources transmit a low-power illumination of a longer perception which generates the amplified image instead of the initial illumination source. The assimilation and retransmission (reradiation) of illumination by organic and inorganic samples is usually the consequence of a permanent physical occurrence, outlined as being fluorescence. When radiation persists long after the agitation illumination has been put out, the phenomenon is said to be phosphorescence [21].

According to Slavík [22], a fluorescence microscope (FM) is an instrument that authorizes the dynamic acquisition of detail based on the spectroscopic characteristics of fluorescent reporter molecules, at very small degrees of resolution, which can be seen by the naked eye. Furthermore, Fluorescence Lifetime Imaging Microscopy (FLIM) has become a potent and widely used instrument to monitor inter and intramolecular dynamics of fluorophore-labelled proteins inside living cells [23]. FM is a widely used study device deployed throughout disciplines in both biomedical and biological sciences [24]. Finally, FM is the main instrument with which to observe cell physiology [25].

In addition, when reflected illumination, and backdrop fluorescence are passed through this category of microscopy, the targeted parts of a particular specimen can be observed in 3D (Fig. 1.8). This is achieved by way of the application of strong illumination sources, like lasers, that can be focused precisely. This process is carried out frequently across specimens.

In most instances, the specimen in question is treated with a well-known fluorescent material such as a fluorophore and is then illuminated through the lens using an improved power source. The importance of fluorophore is to label cells, tissues, and proteins by using a fluorescent marker for analysis by FM [26]. The illuminating light is then assimilated by the fluorophores and leads them to release a prolonged smaller power frequency illumination. This fluorescent illumination can be separated from the surrounding radiation emission with filters created for that specific wavelength, permitting the observer to see exclusively what is fluorescing [27]. Most of the FM employed in biology nowadays are known as epi-fluorescence

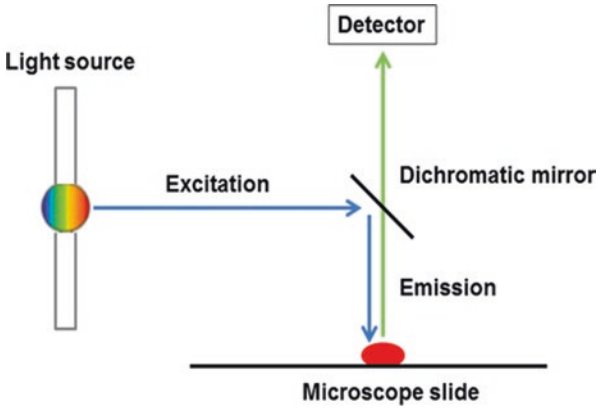

Fig. 1.8 Fundamentals of a fluorescence microscopy

microscopes, meaning that both the agitation and the monitoring (observation) of the fluorescence take place on the specimen. Most FMs use a Xe or Hg arc dismissal (discharge) flashlight for their lighting, as these categories produce a highly intense illumination source. Another class of microscope, corresponds to Total Internal Reflection Fluorescence (TIRF) microscopy, which produces an illuminated light source (e.g., in the range between 50 and 100 nm) located at the interface of the slide itself; this greatly reduces out-of-focus light and improves the ability to identify fluorescent molecules. It has proved to be a critical method in living cell imaging.

1.3 Electron Microscope

Optical microscopes are not able to differentiate any small-scale structure other than the frequency of illumination. For this reason, engineers such as Ernst Ruska and Max Knoll have invented the Electron Microscope (EM). This kind of microscope uses an electron beam as the source of illumination in place of light [28]. This allows for the discerning of the disposition of atoms in materials and obtains atomic level information by way of an electron beam.

1.3.1 Scanning Electron Microscope

An Scanning Electron Microscope (SEM) makes use of a centered beam of high-powered electrons to produce a diversity of indicators on the area of solid samples. Signals are generated from the liaisons between electrons and the specimen, then release the previously unseen details of the said specimen [29].

Figure 1.9 represents a field emission SEM that operates both in variable pressure mode, as well as high vacuum mode; the two functions both use a resolution of

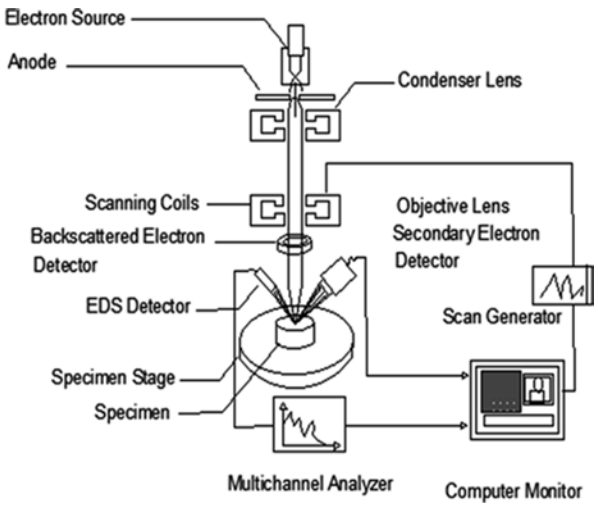

Fig. 1.9 Schematic of a Scanning Emission Microscope

~10 nm. This type of microscope has its characteristics, i.e., it is equipped with (a) an electron detector placed on the lens, (b) an X-ray spectroscopy system made up of the so-called energy dispersion (EDS) that contains drift detectors of Dual silicon (each with an area of 60 mm^2 and a resolution of 123 eV), (c) a cathodoluminescence detector (CL) and (d) with secondary and backscattered electron detectors. In an SEM, images are obtained by scanning the beam showing the signal from an electron detector on a personal computer monitor [30].

An SEM is composed of (a) lenses, a sample chamber, detectors, and a search coil. Secondly, by (b) a source of electrons. For instance, Field Emission Gun (FEG) is made of cerium hexaboride (CeB6) or lanthanum hexaboride (LaB6), and tungsten electron filament (W). Other components of SEMs are Secondary Electron Detector (SED); Energy Scattering Spectroscopy (EDS), and Electron Backscatter Detector (BSD).

One of the characteristics SEMs share is that they always have at least one detector, which is generally of the secondary electron type. Also, most of these instruments have additional detectors. It is understood that the specific abilities of any instrument depend on the detectors that are adapted.

When planning to prepare a specimen for further analysis, its provenance (acquisition) must first be taken into account and of course, its size and whether it will fit into the SEM chamber itself. Secondly, any adjustments are taken into account to avoid charge accumulation in electrically insulating specimens.

Almost all electrically insulating specimens are covered with a significant thin layer of either carbon, gold, or some other metals. In cases in which the elemental analysis of a specimen is crucial or, indeed, a priority, carbon is the most desirable. However, electrically insulating specimens coated with a layer of metal (e.g., gold)

are more effective or safer for applications such as high-resolution electronic imaging. Furthermore, one of the advantages of the electrically insulating specimen is that it can be examined without the use of a conductive coating in an instrument capable of low vacuum operation.

In summary, among the capabilities that these instruments are the following: electrical and metallurgical material failure analysis, polymer and life science, semiconductor design, material identification, forensic science, etc.

1.3.2 Transmission Electron Microscope

A Transmission Electron Microscope (TEM) is an analytical tool permitting visualization and analysis of samples from the lands of micro space (1 μm = 10^{-6} m) to nano space (1 nm = 10^{-9} m). A TEM discloses levels of information and intricacy unreachable by light microscopy due to the fact that it employs a centered beam of high-power electrons. It permits precise microstructural assessment via high-resolution as well as high amplification imaging [31].

The main use of TEM is to process images from a specimen by enlightening the specimen with electrons inside a void and discovering the electrons that are transferred by way of the specimen. By using a TEM, we are able to see all of an atom's column present in crystalline specimens.

A TEM produces a bright-field image (conventional image) of a specimen which can be likened to that of shadow puppetry. For its operation, TEM makes use of a beam of extremely energetic electrons instead of the light of a torch. During operation, through to the specimen being processed, portions of the material deflect or detain electrons more than other portions. Electrons are gathered from below the sample using a phosphorescent monitor (screen). In areas where electrons do not pass through the specimen, the displayed image is obscure, while where electrons cross, the image is brilliant and a range of grays are observed in the middle; which depends greatly on how the electrons interact and are scattered through the sample (Fig. 1.10).

Amplifications of up to 1,000,000× and resolution under 1 nm are commonly reached. Another aspect to consider is that quantitative and qualitative elemental analyses can be provided based on characteristics as minuscule as 1 nm. For instance, for a crystalline stage both the crystal structure, as well as the restrictions of the lattice and the orientation of the specimen can be determined. To our knowledge, inside an atom, there are negatively charged particles called electrons (e−). These, at the same time, cannot be focused with glass lenses compared to photons, which are used by so-called "electromagnets" to focus electrons.

1.4 Scanning Probe Microscope

An Scanning Probe Microscope (SPM) is a tool employed for examining surfaces at a nanoscale level [32]. SPM is employed to produce an exclusive likeness of nanoscale structures and surfaces or maneuvre atoms to displace them in particular