1.1 Bright field microscopy

Bright field Microscopy is the simplest of all the optical micrscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorance of some of the transmitted light in dense areas of the sample. Bright field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright field microscopy image is a dark sample on a bright background, hence the name.

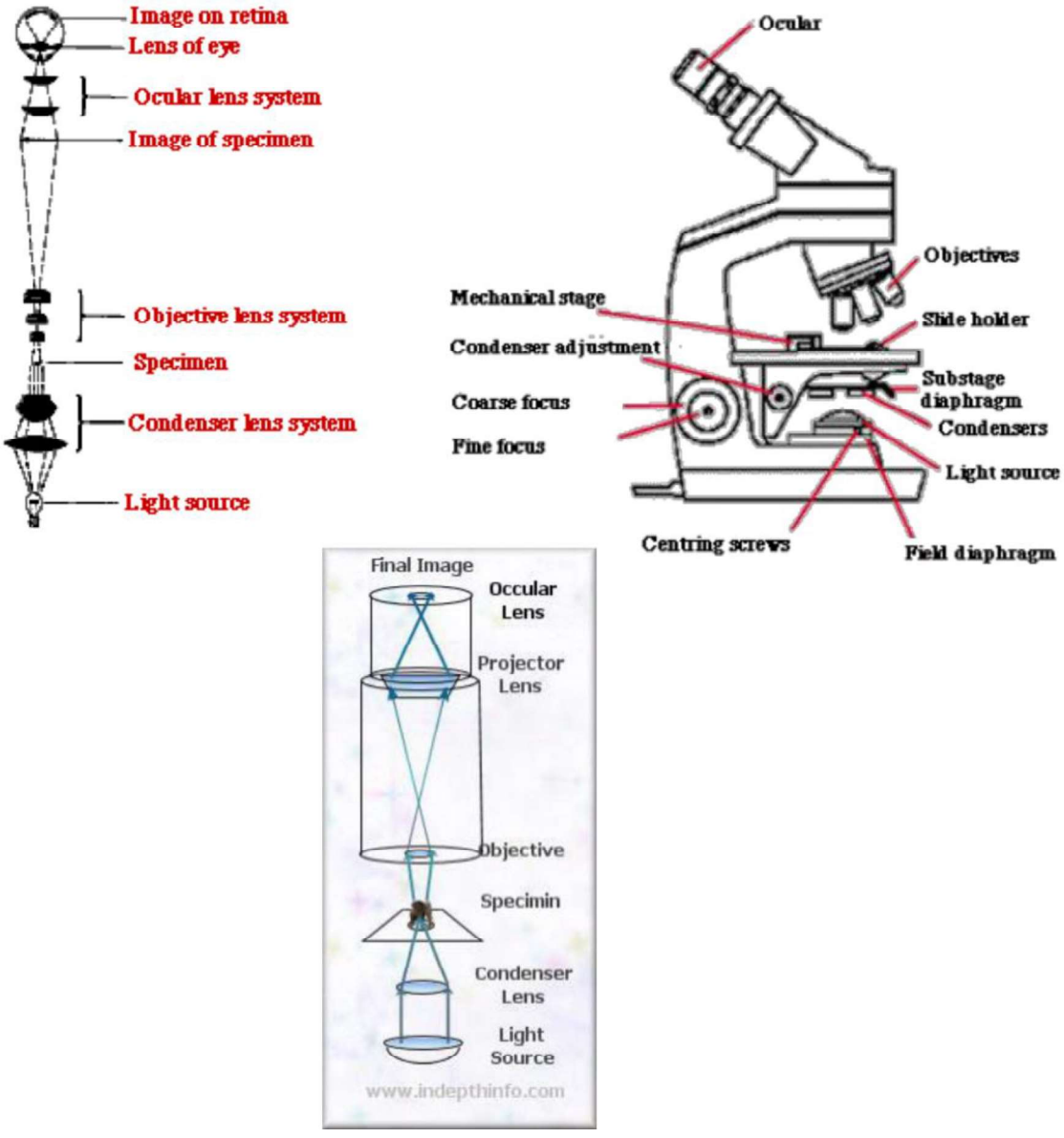

Figure 1 Light path of bright field microscope

Light path - The light path of a bright field microscope is extremely simple, no additional components are required beyond the normal light microscope setup (Fig 1). The light path therefore consists of:

Transillumination light source, commonly a halogen lamp in the microscope stand; A halogen lamp, also known as a tungsten halogen lamp or quartz iodine lamp, is an incandescent lamp that has a small amount of a halogen such as iodine or bromine added. The combination of the halogen gas and the tungsten filament produces a halogen cycle chemical reaction which redeposits evaporated tungsten back onto the filament, increasing its life and maintaining the clarity of the envelope

C**ondenser lens** which focuses light from the light source onto the sample. A condenser is one of the main components of the optical system of many transmitted light compound microscopes. A condenser is a lens that serves to concentrate light from the illumination source that is in turn focused through the object and magnified by the objective lens.

Objective lens : In an optical instrument, the objective is the optical element that gathers light from the object being observed and focuses the light rays to produce a real image. Objectives can be single lenses or mirrors, or combinations of several optical elements. Microscope objectives are characterized by two parameters: magnification and numerical aperture. The typically ranges are 4× , 10x , 40x and 100×. 4. oculars to view the sample image.

An **eyepiece**, or **ocular lens**, is a type of lens that is attached to a variety of optical devices such as microscopes. It is so named because it is usually the lens that is closest to the eye when someone looks through the device. The objective lens or mirror collects light and brings it to focus creating an image. The eyepiece is placed near the focal point of the objective to magnify this image. The amount of magnification depends on the focal length of the eyepiece.

Magnification is the process of enlarging something only in appearance, not in physical size. Typically magnification is related to scaling up visuals or images to be able to see more detail, increasing resolution.

Resolving power is the ability of an imaging device to separate (i.e., to see as distinct) points of an object that are located at a small angular distance..

In optics, the **numerical aperture** (NA) of an optical system is a dimensionless number that characterizes the range of angles over which the system can accept or emit light. In most areas

of optics, and especially in microscopy, the numerical aperture of an optical system such as an objective lens is defined by

NA = n Sin

where n is the index of refraction of the medium in which the lens is working (1.0 for air, 1.33 for pure water, and up to 1.56 for oils; see also list of refractive indices), and θ is the half-angle of the maximum cone of light that can enter or exit the lens. In general, this is the angle of the real marginal ray in the system

1.1.1 Working Performance

Bright field microscopy typically has low contrast with most biological samples as few absorb light to a great extent. Staining is often required to increase contrast, which prevents use on live cells in many situations. Bright field illumination is useful for samples which have an intrinsic colour, for example chloroplasts in plant cells.

Light is first emitted by the light **source** and is directed by the **condenser lens** on to the specimen, which might be a loose object, a prepared plate or almost anything. A microscope can even be applied to small parts of larger objects, though with a bit more difficulty. (The light does not absolutely need to originate below the specimen.)

The light from the specimen then passes through the objective lens. This lens is often selected from among three or four and is the main determinant for the level of magnification. It bends the light rays and in the case of this example sends them to a **projector lens**, which reverses their direction so that when the image reaches the eye it will not appear "upside-down". Not all microscopes have a projector lens, so the viewer may be seeing a reverse image. In these cases, when the slide is moved, it will appear to be moving in the opposite direction to the viewer.

The light rays then travel to the oracular lens or "eye piece". This is often a 10X magnification lens, meaning it magnifies the magnified image an additional ten times. The image is then projected into the eye. It is very seldom that a specimen is in focus the moment it is placed beneath a microscope. This means that some adjustment will have to be made. Unlike in telescopes, the focal length between lenses remains constant when adjusting the focus. The lens apparatus is brought closer to or further from the object. The focus adjustment is often along the neck of the tube containing the lenses, but it might just as well move the slide up and down. The best way to make this adjustment is to make a course adjustment so that it is too close to the

object and then back off with the fine adjustment2. This helps to ensure that the specimen is not inadvertently smashed by the lens.

1.1.2 Advantages

- The name "brightfield" is derived from the fact that the specimen is dark and contrasted by the surrounding bright viewing field. Simple light microscopes are sometimes referred to as bright field microscopes.
- Brightfield microscopy is very simple to use with fewer adjustments needed to be made to view specimens.
- Some specimens can be viewed without staining and the optics used in the brightfield technique don't alter the color of the specimen.
- It is adaptable with new technology and optional pieces of equipment can be implemented with brightfield illumination to give versatility in the tasks it can perform.

1.1.3 Disadvantages

Certain disadvantages are inherent in any optical imaging technique.

- By using an aperture diaphragm for contrast, past a certain point, greater contrast adds distortion. However, employing an iris diaphragm will help compensate for this problem.
- Brightfield microscopy can't be used to observe living specimens of bacteria, although when using fixed specimens, bacteria have an optimum viewing magnification of 1000x.
- Brightfield microscopy has very low contrast and most cells absolutely have to be stained to be seen; staining may introduce extraneous details into the specimen that should not be present.
- Also, the user will need to be knowledgeable in proper staining techniques.
- Lastly, this method requires a strong light source for high magnification applications and intense lighting can produce heat that will damage specimens or kill living microorganisms.

1.2Phase contrast microscopy

Phase contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations.

When light waves travels through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the

medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength dependent and may give rise to colors. Photographic equipment and the human eye are only sensitive to amplitude variations. Without special arrangements, phase changes are therefore invisible. Yet, often these changes in phase carry important information.

1.2.1 History and Background Information

Frits Zernike, a Dutch physicist and mathematician, built the first phase contrast microscope in 1938.

It took some time before the scientific community recognized the potential of Zernike's

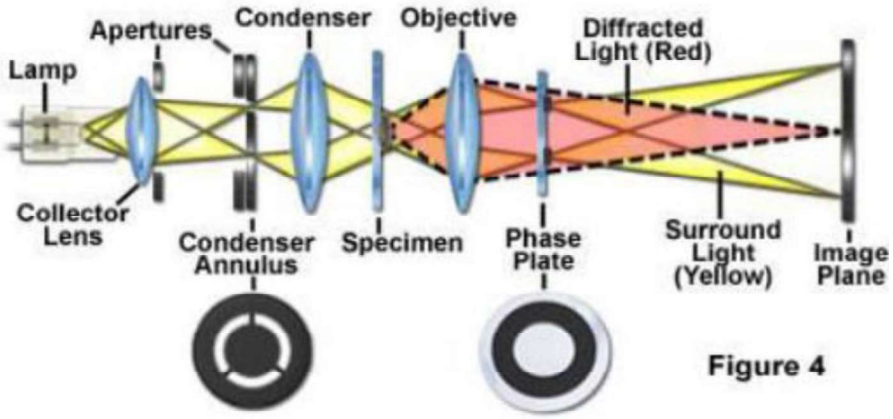

Figure 4

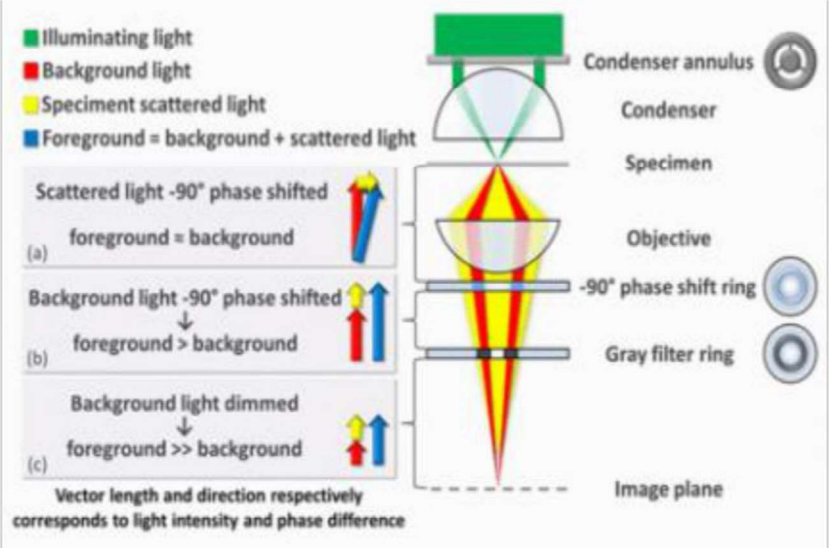

Figure 2 Phase contrast microscope

discovery; he won the Nobel Prize in 1953 and the German-based company Zeiss began manufacturing his phase contrast microscope during World War II. .

1.2.2 Working Principle

The basic principle to make phase changes visible in phase contrast microscopy is to separate the illuminating background light from the specimen scattered light, which make up the foreground details, and to manipulate these differently.

The ring shaped illuminating light (green) that passes the condenser annulus is focused on the specimen by the condenser. Some of the illuminating light is scattered by the specimen (yellow). The remaining light is unaffected by the specimen and form the background light (red). When observing unstained biological specimen, the scattered light is weak and typically phase shifted by -90° — relative to the background light. This leads to that the foreground (blue vector) and the background (red vector) nearly have the same intensity, resulting in a low image contrast (a). In a phase contrast microscope, the image contrast is improved in two steps. The background light is phase shifted -90° by passing it through a phase shift ring. This eliminates the phase difference between the background and the scattered light, leading to an increased intensity difference between foreground and background (b). To further increase contrast, the background is dimmed by a gray filter ring (c). Some of the scattered light will be phase shifted and dimmed by the rings. However, the background light is affected to a much greater extent, which creates the phase contrast effect (Fig 2).

The above describes negative phase contrast. In its positive form, the background light is instead phase shifted by +90°. The background light will thus be 180° out of phase relative to the scattered light. This results in that the scattered light will be subtracted from the background light in (b) to form an image where the foreground is darker than the background.

12.3 Applications in Microscopy

The possible applications of Zernike's phase contrast microscope in microscopy are evident in the fields of molecular and cellular biology, microbiology and medical research.

Specimens that can be observed and studied include live microorganisms such as protozoa, erythrocytes, bacteria, molds and sperm, thin tissue slices, lithographic patterns, fibers, glass fragments and sub-cellular particles such as nuclei and organelles.

1.2.4 Advantages

The advantages of the phase contrast microscope include:

- The capacity to observe living cells and, as such, the ability to examine cells in a natural state
- Observing a living organism in its natural state and/or environment can provide far more information than specimens that need to be killed, fixed or stain to view under a microscope
- High-contrast, high-resolution images
- Ideal for studying and interpreting thin specimens
- Ability to combine with other means of observation, such as fluorescence
- Modern phase contrast microscopes, with CCD or CMOS computer devices, can capture photo and/or video images
- In addition, advances to the phase contrast microscope, especially those that incorporate technology, enable a scientist to hone in on minute internal structures of a particle and can even detect a mere small number of protein molecules.

1.2.5 Disadvantages

- Disadvantages and limitations of phase contrast:
- Annuli or rings limit the aperture to some extent, which decreases resolution
- This method of observation is not ideal for thick organisms or particles
- Thick specimens can appear distorted
- Images may appear grey or green, if white or green lights are used, respectively, resulting in poor photomicrography
- Shade-off and halo effect, referred to a phase artifacts
- Shade-off occurs with larger particles, results in a steady reduction of contrast moving from the center of the object toward its edges
- Halo effect, where images are often surrounded by bright areas, which obscure details along the perimeter of the specimen
- Modern advances and techniques provide solutions to some of these confines, such as the halo effect.
- Anodized phase contrast utilizes amplitude filters that contain neutral density films to minimize the halo effect. Essentially, this is attempting to reverse the definition achieved through phase contrast annuli, but the halo effect can never be eliminated completely.

- The pros that phase contrast has brought to the field of microscopy far exceed its limitations. This is easily seen with the myriad of advances in the fields of cellular and microbiology as well as in medical and veterinary sciences.

1.2.6 Conclusion

The **phase contrast microscope** opened up an entire world of microscopy, providing incredible definition and clarity of particles never seen before.

1.3 Fluorescence microscope

Figure 3 An upright fluorescence microscope (Olympus BX61) with the fluorescent filter cube turret above the objective lenses, coupled with a digital camera.

A **fluorescence microscope** is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances. The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image (Fig 3).

1.3.1 Principle

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker

emitted fluorescence through the use of a spectral emission filter. Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror (or dichroic beamsplitter), and the emission filter (see figure below). The filters and the dichroic are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen.In this manner, the distribution of a single fluorophore (color) is imaged at a time. Multi-color images of several types of fluorophores must be composed by combining several single-color images.

Most fluorescence microscopes in use are epifluorescence microscopes, where excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e. through the objective). These microscopes are widely used in biology and are the basis for more advanced microscope designs, such as the confocal microscope and the total internal reflectio fluorescence microscope (TIRF).

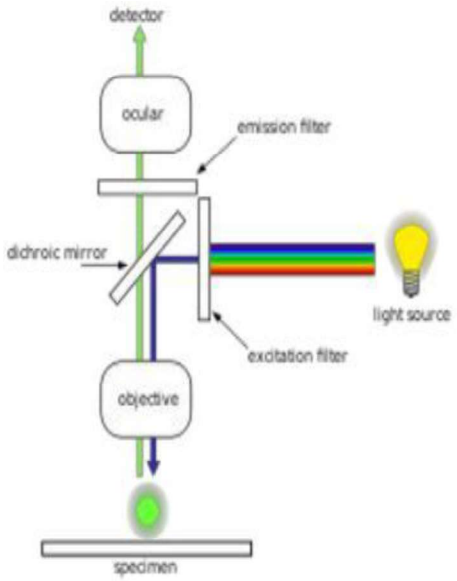

Figure 4 Schematic of a fluorescence microscope.

The majority of fluorescence microscopes, especially those used in the life sciences, are of the epifluorescence design shown in the diagram. Light of the excitation wavelength is focused onthe specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greatest sensitivitywill have a very high numerical aperture. Since most of the excitation light is

transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and the epifluorescence method therefore gives a high signal to noise ratio. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light (Fig 3).

1.3.2 Light sources

Fluorescence microscopy requires intense, near-monochromatic, illumination which some widespread light sources, like halogen lamps cannot provide. Four main types of light source are used, including xenon arc lamps or mercury-vapor lamps with an excitation filter, lasers, supercontinuum sources, and high-power LEDs. Lasers are most widely used for more complex fluorescence microscopy techniques like confocal microscopy and total internal reflection fluorescence microscopy while xenon lamps, and mercury lamps, and LEDs with a dichroic excitation filter are commonly used for widefield epifluorescence microscopes (Fig 4).

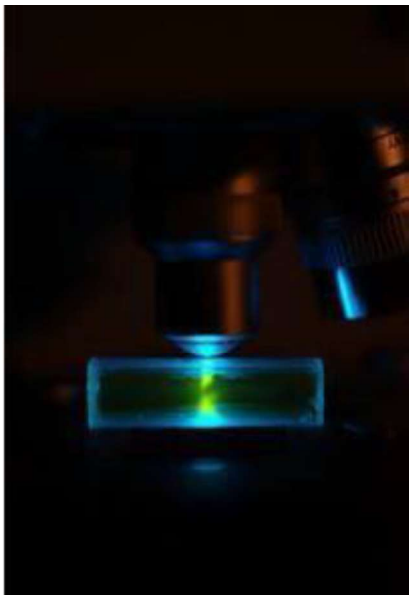

Figure 5 Light source

1.3.3 Sample preparation

A sample of herring sperm stained with SYBR green in a cuvette illuminated by blue light in an epifluorescence microscope. The SYBR green in the sample binds to the herring sperm DNA and, once bound, fluoresces giving off green light when illuminated by blue light.

In order for a sample to be suitable for fluorescence microscopy it must be fluorescent. There are several methods of creating a fluorescent sample; the main techniques are labelling with

fluorescent stains or, in the case of biological samples, expression of a fluorescent protein. Alternatively the intrinsic fluorescence of a sample (i.e., autofluorescence) can be used.[1] In the life sciences fluorescence microscopy is a powerful tool which allows the specific and sensitive staining of a specimen in order to detect the distribution of proteins or other molecules of interest. As a result there is a diverse range of techniques for fluorescent staining of biological samples

1.3.4 Biological fluorescent stains

Many fluorescent stains have been designed for a range of biological molecules. Some of these are small molecules which are intrinsically fluorescent and bind a biological molecule of interest. Major examples of these are nucleic acid stains like DAPI and Hoechst (excited by UV wavelength light) and DRAQ5 and DRAQ7 (optimally excited by red light) which all bind the minor groove of DNA, thus labelling the nuclei of cells. Others are drugs or toxins which bind specific cellular structures and have been derivatised with a fluorescent reporter. A major example of this class of fluorescent stain is phalloidin which is used to stain actin fibres in mammalian cells.

There are many fluorescent molecules called fluorophores or fluorochromes such as fluorescein, Alexa Fluors or DyLight 488, which can be chemically linked to a different molecule which binds the target of interest within the sample.

1.4 Electron microscope

An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects. A transmission electron microscope can achieve better than 50 pm resolution[1] and magnifications of up to about 10,000,000x whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000x. Transmission electron microscopes use electrostatic and electromagnetic lenses to control the electron beam and focus it to form an image. These electron optical lenses are analogous to the glass lenses of an optical light microscope. Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals. Industrially, electron microscopes are often used for quality control and failure analysis. Modern electron

microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the image.

1.4.1Types

14.1.1 Transmission electron microscope (TEM) The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to illuminate the specimen and create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 40018keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide.

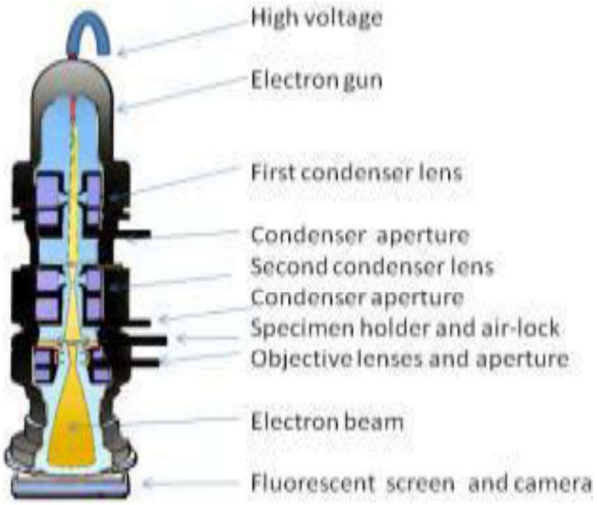

Figure 6 Transmission electron microscope

Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a digital camera. The image detected by the digital camera may be displayed on a monitor or computer.

The resolution of TEMs is limited primarily by spherical aberration, but a new generation of aberration correctors have been able to partially overcome spherical aberration to increase

resolution. Hardware correction of spherical aberration for the high-resolution transmission electron microscopy (HRTEM) has allowed the production of images with resolution below 0.5 angstrom (50 picometres) and magnifications above 50 million times. The ability to determine the positions of atoms within materials has made the HRTEM an important tool for nano-technologies research and development.

Transmission electron microscopes are often used in electron diffraction mode. The advantages of electron diffraction over X-ray crystallography are that the specimen need not be a single crystal or even a polycrystalline powder, and also that the Fourier transform reconstruction of the object's magnified structure occurs physically and thus avoids the need for solving the phase problem faced by the X-ray crystallographers after obtaining their X-ray diffraction patterns of a single crystal or polycrystalline powder.

The major disadvantage of the transmission electron microscope is the need for extremely thin sections of the specimens, typically about 100 nanometers. Biological specimens are typically required to be chemically fixed, dehydrated and embedded in a polymer resin to stabilize them sufficiently to allow ultrathin sectioning. Sections of biological specimens, organic polymers and similar materials may require special treatment with heavy atom labels in order to achieve the required image contrast.

1.4.1.2 Scanning electron microscope (SEM)

The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated. In the SEM image of an ant shown below and to the right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs.

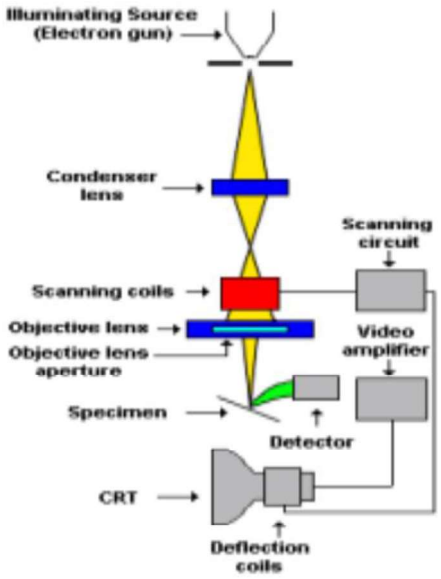

Figure 7 Scanning electron microscope

Generally, the image resolution of an SEM is at least an order of magnitude poorer than that of a TEM. However, because the SEM image relies on surface processes rather than transmission, it is able to image bulk samples up to many centimeters in size and (depending on instrument design and settings) has a great depth of field, and so can produce images that are good representations of the three-dimensional shape of the sample. Another advantage of SEM is its variety called environmental scanning electron microscope (ESEM) can produce images of sufficient quality and resolution with the samples being wet or contained in low vacuum or gas. This greatly facilitates imaging biological samples that are unstable in the high vacuum of conventional electron microscopes.

1.4.2 Color

In their most common configurations, electron microscopes produce images with a single brightness value per pixel, with the results usually rendered in grayscale. However, often these images are then colorized through the use of feature-detection software, or simply by hand editing using a graphics editor. This may be done to clarify structure or for aesthetic effect and generally does not add new information about the specimen.

1.4.3 Sample preparation

Materials to be viewed under an electron microscope may require processing to produce a suitable sample.

The technique required varies depending on the specimen and the analysis required:

- Chemical fixation – for biological specimens aims to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide.

- Negative stain – suspensions containing nanoparticles or fine biological material (such as viruses and bacteria) are briefly mixed with a dilute solution of an electron-opaque solution such as ammonium molybdate, uranyl acetate (or formate), or phosphotungstic acid. This mixture is applied to a suitably coated EM grid, blotted, then allowed to dry. Viewing of this preparation in the TEM should be carried out withoutdelay for best results. The method is important in microbiology for fast but crude morphological identification, but can also be used as the basis for high resolution 3D reconstruction using EM tomography methodology when carbon films are used for support. Negative staining is also used for observation of nanoparticles.

- Cryofixation – freezing a specimen so rapidly, in liquid ethane, and maintained at liquid nitrogen or even liquid helium temperatures, so that the water forms vitreous (noncrystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy of vitreous sections (CEMOVIS), it is now possible to observe samples from virtually any biological specimen close to its native state.[citation needed]

- Dehydration – or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins. Also freeze drying.

- Embedding, biological specimens – after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. To do this the tissue is passed through a 'transition solvent' such as propylene oxide (epoxypropane) or acetone and then infiltrated with an epoxy resin such as Araldite, Epon, or Durcupan; tissues may also be embedded directly in water-miscible acrylic resin. After the resin has been polymerized (hardened) the sample is thin sectioned (ultrathin sections) and stained – it is then ready for viewing.

- Embedding, materials – after embedding in resin, the specimen is usually ground and polished to a mirror-like finish using ultra-fine abrasives. The polishing process must be performed carefully to minimize scratches and other polishing artifacts that reduce image quality.

- Metal shadowing – Metal (e.g. platinum) is evaporated from an overhead electrode and applied to the surface of a biological sample at an angle. The surface topography results in variations in the thickness of the metal that are seen as variations in brightness and contrast in the electron microscope image.

- Replication – A surface shadowed with metal (e.g. platinum, or a mixture of carbon and platinum) at an angle is coated with pure carbon evaporated from carbon electrodes at right angles to the surface. This is followed by removal of the specimen material (e.g. in an acid bath, using enzymes or by mechanical separation) to produce a surface replica that records the surface ultrastructure and can be examined using transmission electron microscopy.

- Sectioning – produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce ultra-thin sections about 60–90 nm thick. Disposable glass knives are also used because they can be made in the lab and are much cheaper.

- Staining – uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens can be stained "en bloc" before embedding and also later after sectioning. Typically thin sections are stained for several minutes with an aqueous or alcoholic solution of uranyl acetate followed by aqueous lead citrate.

- Freeze-fracture or freeze-etch – a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixation), then fractured by breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold fractured surface (sometimes "etched" by increasing the temperature to about −100 °C for several minutes to let some ice sublime) is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The still-floating replica is thoroughly washed free from residual chemicals, carefully fished up on fine grids, dried then viewed in the TEM.

- Ion beam milling – thins samples until they are transparent to electrons by firing ions (typically argon) at the surface from an angle and sputtering material from the surface. A subclass of this is focused ion beam milling, where gallium ions are used to produce an electron transparent membrane in a specific region of the sample, for example through a device within a microprocessor. Ion beam milling may also be used for cross-section polishing prior to SEM analysis of materials that are difficult to prepare using mechanical polishing.

- Conductive coating – an ultrathin coating of electrically conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done toprevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. The coating materials include gold, gold/palladium, platinum, tungsten, graphite, etc.

- Earthing – to avoid electrical charge accumulation on a conductive coated sample, it is usually electrically connected to the metal sample holder. Often an electrically conductive adhesive is used for this purpose.

1.4.4 Disadvantages

Electron microscopes are expensive to build and maintain, but the capital and running costs of confocal light microscope systems now overlaps with those of basic electron microscopes. Microscopes designed to achieve high resolutions must be housed in stable buildings (sometimes underground) with special services such as magnetic field cancelling systems.

The samples largely have to be viewed in vacuum, as the molecules that make up air would scatter the electrons. An exception is the environmental scanning electron microscope, which allows hydrated samples to be viewed in a low-pressure (up to 20 Torr or 2.7 kPa) and/or wet environment.

Scanning electron microscopes operating in conventional high-vacuum mode usually image conductive specimens; therefore non-conductive materials require conductive coating (gold/palladium alloy, carbon, osmium, etc.). The low-voltage mode of modern microscopes makes possible the observation of non-conductive specimens without coating. Non-conductive materials can be imaged also by a variable pressure (or environmental) scanning electron microscope.

Small, stable specimens such as carbon nanotubes, diatom frustules and small mineral crystals (asbestos fibres, for example) require no special treatment before being examined in the electron microscope. Samples of hydrated materials, including almost all biological specimens have to be prepared in various ways to stabilize them, reduce their thickness (ultrathin sectioning) and increase their electron optical contrast (staining).