

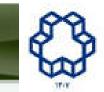
K. N. Toosi University of Technology Faculty of Materials Science and Engineering



Materials Characterization Methods

Tenth Session (Optical Microscope)

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#### History

- ✓ The earliest microscopes were single lens magnifying glasses with limited magnification, which date at least as far back as the widespread use of lenses in eyeglasses in the 13th century.
- ✓ Compound microscopes first appeared in Europe around 1620 including one demonstrated by Cornelis Dribble in London (around 1621) and one exhibited in Rome in 1624. The actual inventor of the compound microscope is unknown although many claims have been made over the years. These include a claim 35 years after they appeared by Dutch spectacle-maker Johannes Zacharias Sen that his father, Zacharias Janssen, invented the compound microscope and/or the telescope as early as 1590.

#### History

Another claim is that Janssen's competitor, Hans Lippert (who applied for the first telescope patent in 1608) also invented the compound microscope. Galileo Galilei is sometimes cited as a compound microscope inventor. After 1610, he found that he could close focus his telescope to view small objects, such as flies, close up and/or could look through the wrong end in reverse to magnify small objects.



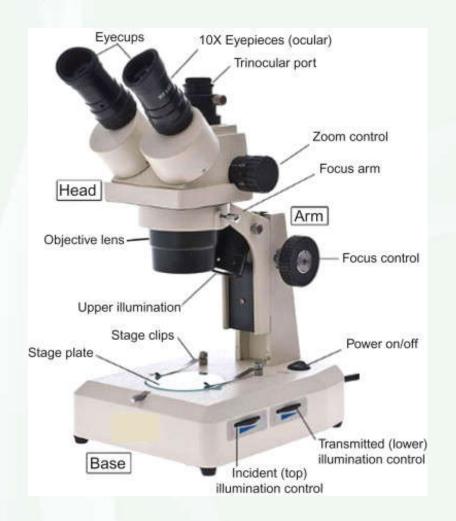
#### History

After seeing the compound microscope built by Drebbel exhibited in Rome in 1624, Galileo built his own improved version. In 1625, Giovanni Faber coined the name microscope for the compound microscope Galileo. Galileo had called it the "occhiolino" or "little eye". Faber coined the name from the Greek words  $\mu$ ukpóv (micron) meaning "small", and  $\sigma$ ko $\pi$ ɛĩv (skopein) meaning "to look at", a name meant to be analogous with "telescope".

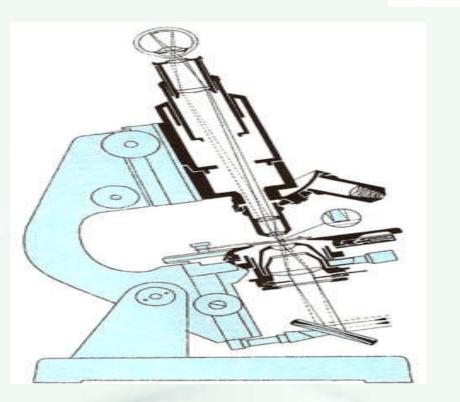


One of the first techniques used to study the topography of a surface is optical microscopy, also called light microscopy. An optical microscope usually has a single eyepiece which can often be fitted with a camera for photography. In contrast another device called a stereomicroscope uses a light microscope with dual eyepieces. The examined samples can be embedded in a holding material (Bakelite or epoxy, usually) called a metallurgical mount. This type of specimen configuration provides a planar cross-sectional view of a fracture surface along with adjacent material for evaluation.



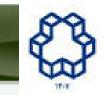


#### Stereomicroscope



#### **Optical Microscopy**





Conventional optical microscopes have a resolution limited by the size of submicron particles approaching the wavelength of visible light (400–700 nm). The two types of available optical microscope, which depend on the type of light exposure, include:

1 -Transmission: Beam of light passes through the sample
2 -Reflection: Beam of light reflected off the sample surface. An example is the polarizing or petrographic microscope for which the samples are usually fine powder or thin slices (transparent). Another example is the metallurgical or reflected light microscope which is used for the surfaces of materials, especially opaque ones.



#### ✓ Bright-Field Microscope

This is the simplest and most common type of optical microscope. It uses a bright light source to illuminate the sample, and produces a dark image on a bright background. It is used to observe the structure and morphology of a wide range of specimens, such as cells, tissues, and microorganisms.





### **Types of Optical Microscopes**

#### ✓ Dark-Field Microscope

This type of microscope uses a special condenser lens that directs light at an oblique angle onto the sample, causing the sample to appear bright on a dark background. It is used to observe samples that do not absorb or transmit much light, such as bacteria, viruses, and nanoparticles.





#### **Types of Optical Microscopes**

#### ✓ Phase-Contrast Microscope

This type of microscope is used to observe transparent samples, such as living cells or bacteria, which are difficult to see using traditional bright-field microscopy. It produces images by detecting the differences in the refractive index of the sample, rather than differences in light absorption.





### **Types of Optical Microscopes**

#### ✓ Fluorescence Microscope

This type of microscope uses a light source that excites fluorescent molecules within the sample, causing them to emit light of a different color. It is used to observe samples that have been labeled with fluorescent dyes or antibodies, such as cells and tissues.





### **Types of Optical Microscopes**

#### ✓ Super-Resolution Microscope

This type of microscope uses various techniques to overcome the diffraction limit of light, allowing it to achieve a resolution beyond that of traditional optical microscopy. It is used to observe structures and molecules at the nanoscale, such as proteins, viruses, and cellular organelles.





### **Types of Optical Microscopes**

#### ✓ Confocal Microscope

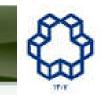
This type of microscope uses a laser to scan a sample point by point, and creates a 3D image by stacking the individual images together. It is used to observe thick or complex samples, such as tissues and embryos, and can produce highresolution images with minimal background noise.





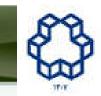
Materials Science: Optical microscopy is used to study the microstructure of materials such as metals, polymers, ceramics, and composites. It enables researchers to understand the relationship between the structure and properties of materials and to develop new materials with desired properties.





 Biological Research: Optical microscopy is extensively used in biological research to visualize biological specimens such as cells, tissues, and microorganisms. It enables researchers to observe and study the internal structure and function of these specimens, which is crucial for understanding biological processes and developing new medical treatments.





 Semiconductor Industry: Optical microscopy is used in the semiconductor industry to analyze and optimize the fabrication process of semiconductor devices. It enables researchers to observe defects and impurities in semiconductor materials and to optimize the performance of semiconductor devices such as transistors and diodes.





Forensic Science: Optical microscopy is used in forensic science to analyze trace evidence such as fibers, hairs, and fingerprints. It enables forensic investigators to identify and compare samples and to provide evidence in criminal investigations.



#### Images

- An optical microscope utilizes standard light-sensitive cameras to generate a micrograph. In the past, photographic film was commonly employed to capture these images.
- ✓ The magnification power of a compound optical microscope relies on the combination of the ocular and objective lenses. It is determined by multiplying the powers of these lenses. For instance, when a 10x ocular lens and a 100x objective lens are used together, the resulting magnification is 1000x.

#### Images

 ✓ Advancements in technology have revolutionized the process, enabling the use of CMOS and charge-coupled device (CCD) cameras to capture digital images with optical microscopes. Consequently, these digital microscopes can project the image onto a computer screen in real time, facilitating the examination of samples. This eliminates the need for eyepieces and enhances convenience.



 Limited Resolution: The resolution of an optical microscope is limited by the diffraction of light, which means that it cannot resolve details smaller than approximately half the wavelength of the light used.





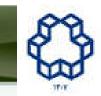
Limited Depth of Field: The depth of field of an optical microscope is limited, meaning that only a narrow section of the sample can be in focus at any given time. This can make it difficult to image thick or 3D samples, and can result in images with blurred or out-of-focus regions.





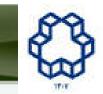
Limited Contrast: Some samples, particularly those that are transparent or have a similar refractive index to their surroundings, can be difficult to see using optical microscopy. This can limit the ability of researchers to observe and analyze certain types of samples.





Photobleaching & Phototoxicity: Fluorescence microscopy, in particular, can be limited by photobleaching, which is the irreversible destruction of fluorescent molecules due to light exposure, and phototoxicity, which is the damage to living cells or tissues due to prolonged light exposure.





Sample Preparation: Preparing samples for optical microscopy can be time-consuming and may require specialized techniques or equipment. Some samples may also be damaged or altered during the preparation process, which can affect the accuracy and reliability of the observations.

