

**Structural Biology**  
**Prof. Saugata Hazara**  
**Department of Biotechnology**  
**Indian Institute of Technology - Roorkee**

**Lecture – 36**  
**Introduction to Microscopy**

Hi everyone, welcome again to the course on structural biology. We are continuing with structural Biology techniques. We talked about x-ray and NMR. Today, we will start a module where we will talk about cryo-electron microscopy and other high-resolution techniques.

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Microscopy Electron Cryogenic vitreous X Ray Cryo

**Cryo Electron Microscopy:**

**Cryogenic electron microscopy (cryo-EM)** is an electron microscopy (EM) technique applied on samples cooled to cryogenic temperatures and embedded in an environment of vitreous water

An aqueous sample solution is applied to a grid-mesh and plunge-frozen in liquid ethane or a mixture of liquid ethane and propane

While development of the technique began in the 1970s, recent advances in detector technology and software algorithms have allowed for the determination of biomolecular structures at near-atomic resolution



Swagathi

Cryo-electron microscopy or cryogenic electron microscopy, or in short cryo-EM is an electron microscopy technique applied on samples cooled to cryogenic temperatures and embedded in an environment of vitreous water. So look at the terms here. This is microscopy. This is an electron microscope. The source is the electron and applied on the sample, which is cooled to cryogenic temperature and embedded in an environment of vitreous water.

An aqueous sample solution is applied to a grid mesh and plunge frozen in liquid ethane or liquid ethane and propane mixture. For both NMR and Cryo, we are applying an aqueous sample. So, an aqueous sample solution was applied to the grid mesh. So, grid mesh is kind of immobilized and plunge-frozen in liquid ethane. While the development of the technique began in the 1970's recent advances in the detector technology and software algorithms are allowed to determine biomolecular structures at near-atomic resolution.

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**Cryo Electron Microscopy:**

This has attracted wide attention to the approach as an alternative to **X-ray crystallography** or **NMR spectroscopy** for macromolecular structure determination without the need for crystallization

In 2017, the Nobel Prize in Chemistry was awarded to **Jacques Dubochet, Joachim Frank, and Richard Henderson** "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution

*Nature Methods* also named cryo-EM as the "Method of the Year" in 2016



Continuing with the information about cryo-electron microscopy, this had attracted wide attention to the approach as an alternative to x-ray crystallography or NMR Spectroscopy. And one of the biggest things is you do not need a crystal. In 2017 the Nobel Prize in chemistry was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution. Also, nature methods named cryo-EM as the method of the year in 2016.

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## Some Basics:

What is microscopy and how microscopy works

History of microscopy

Simple and compound microscope

Types of microscope

Electron microscope

Transmission and Scanning electron microscope



So, basic questions are there. So we talked about microscopy, how a microscope works, and the history of the microscope a little bit about how it developed? How from starting with the light microscope coming to the electron microscope? What is the electron microscope? How does that go to the cryo-electron microscope? We will talk about simple and compound microscopes, types of microscope, and electron microscope.

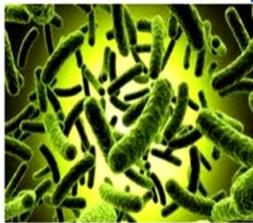
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## What we could observe:

We look to the outer limits of the universe..

We look at microbes

We look at Virus Particles



We look to the outer limits of the universe. We look at microbes bacteria. We look at viruses. We look at chromosomes. Some of them take our limits, what we could see in the normal eye.

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## Seeing through Normal Eye:



Normal eye could not see the objects less than 100 micrometer in diameter

The size of bacteria varies from 1-5 micrometer

The size of viruses ranges from 25-350 nm

- The hand lenses provide us the enlargement of 2 to 10x

Technically they are the simplest microscope

The normal eye could not see an object less than 100 micrometers in diameter. So it is the limit of the normal eye. The size of bacteria varies from 1 to 5 micrometers. The size of viruses ranges from 25 to 350 nanometers, even smaller. The hand lenses allow us to enlarge 2 to 10x. What do I mean by this? I mean, by this, you make a lens and the lens is a tool that would enlarge the eye's power. The lens is helping you to magnify the enlargement of the object. This is the basic concept of a microscope. Something that helps your eye sees by enlarging that object that is not within the limit of your eye. You will say that that is a basic microscope.

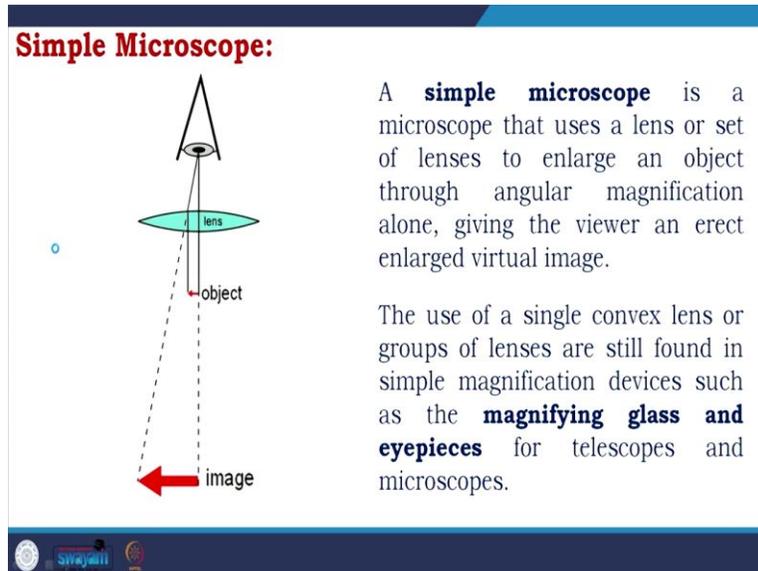
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## Light Microscope:

- The optical microscope, often referred to as light microscope, is a type of microscope which uses **visible light** and a **system of lenses** to magnify images of small samples

The optical microscope, often referred to as a light microscope, uses visible light and a lens system to magnify small sample images. So what started with one lens? Now people have made a little complicated system with different lenses.

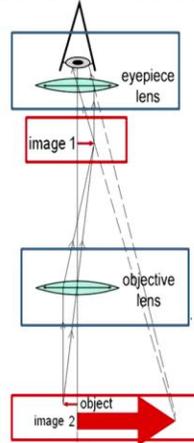
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The simple microscope is a microscope that uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewers an erect, enlarged virtual image. The uses of a single convex lens or group of lenses are still found in simple magnification devices such as the magnifying glass and eyepiece is for telescope and microscope.

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## Compound Microscope:



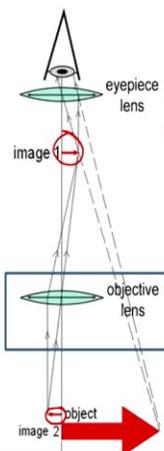
A **compound microscope** is a microscope which uses a lens close to the object being viewed to collect light which focuses a real image of the object inside the microscope

That image is then magnified by a second lens or group of lenses that gives the viewer an enlarged virtual image of the object

A compound microscope is a microscope that uses a lens close to the object being viewed to collect light, focusing on a real image of the object inside the microscope. So there is one lens, and there is another eyepiece lens and objective lens. So, you use a combination of an eyepiece lens and an objective lens. So that image is then magnified by a second lens or group of lenses that give the viewer an enlarged view virtual image of the object. This is the objective lens creating image one. This is the eyepiece lens creating the further enlarge object.

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## Objective Lens:



Role:

- A) To gather light rays coming from any point of the object
- B) To unite the light in a point of image
- C) To magnify the image

What is about the objective lens role? To gather light rays coming from any object point, you have the object. The function of the objective lens is to gather light rays coming from any point

of the object. To unite the light in a point of the image, it goes and united in the image and magnifies. This magnification is the function of the objective lenses.

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**Aberrations:**

- **Spherical aberration:**  
Light rays hitting periphery will be more refracted than the rays hitting centre of lens.
- **Chromatic Aberrations:**  
White light of passing through simple lens, each wavelength will be refracted to different extent.  
**Blue brought to a shorter focus than red, results in a un-sharp image with color fringes.**



What is an aberration? There are two types of aberration. One is spherical aberration. Light rays hitting the periphery will be more refracted than those hitting the central lens. And there is chromatic aberration, white light passing through a simple lens, each wavelength will be refracted to a different extent. As we know, the blue brought to a shorter focus than red resulting in an un-sharp image with color fringes.

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**Objective Lens:**

Three types of objectives

- a) Achromatic: Simplest in construction and adequate for most of the purposes
- b) Fluorite: Aberrations are largely removed by this type of objectives
- c) Apochromatic: Most corrected in terms of aberration

There are three types of objective lenses achromatic, which are simplest in construction and adequate for most purposes. Fluorite: aberrations are largely removed by this type of objective. Apochromatic: most corrected in terms of aberration.

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**Eyepiece Lens:**

Role:

- A) To magnify the real image of the object from the objective lens
- B) To correct the defects of the objective

Eyepiece Lens: Role to magnify the real image to the object from the objective lens and correct the objective's defects. So it takes to magnify. So this is the real object it comes to image 1. So, the role of the eyepiece lens is to take image one and enlarge it, correcting the defects of the objective lens. What is the usefulness of a compound microscope? The use of a compound objective and eyepiece combination allows for much higher magnification and also helps in removing the aberrations.

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**Optical Microscope:**

- Resolving Power
- Numerical Aperture
- Magnification

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In an optical microscope, the functionality depends on the resolving power, numerical aperture, and magnification.

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**Resolving Power:**

The power of an objective able to separate distinctly two adjacent points

**It is dependent on,**

- Wavelength of the light used
- Numerical aperture of the lens

**Larger NA means greater resolving power of the objective and finer detail could be studied**

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What is the resolving power? The power of an objective can separate two adjacent points. Two adjacent points are very close, so you use a lens, and you cannot separate, and you use a lens you could separate that the resolving power. So resolving power depends on the wavelength of the light, the numerical aperture of the lens. A larger numerical aperture means greater resolving power of the objective, and final detail could be studied.

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### Resolving Power:

#### Features:

It is the ability to differentiate two close points as separate

The resolving power of human eye is 0.25 mm

- The light microscope can separate dots that are 0.25 $\mu$ m apart
- The electron microscope can separate dots that are 0.5nm apart

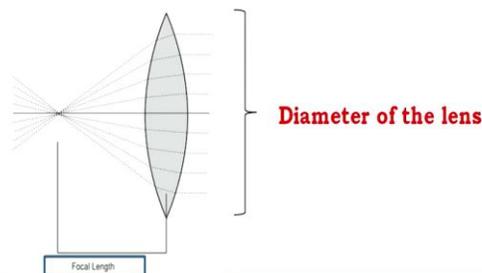
The features for resolving power: It can differentiate two close points as separate. The resolving power of the human eye is 0.25 millimeters. The light microscope can separate dots that are 0.25 micrometer apart. The electron microscope can separate dots that are 0.5 nanometers apart. So now you would understand the difference between a light microscope and an electron microscope. Why does an electron microscope have so high resolving power? We will take a look in our further analysis.

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### Numerical aperture(NA):

The numerical aperture of a lens is the ratio of the diameter of the lens to its focal length

NA can be decreased by decreasing the amount of light that passes through a lens



Numerical aperture NA: The numerical aperture of a lens is the ratio of the diameter of the lens to its focal length. So, if you see this, the focal length from the point the object to the lens and numerical aperture can be decreased by decreasing the amount of light passing through the lens.

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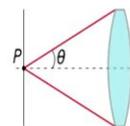
**NUMERICAL APARTURE:**

Is a dimensionless number that characterizes the range of angles over which the system can accept or emit light

It is the acceptance cone of an objective (and hence its light-gathering ability and resolution)

$$NA = n \sin \theta$$

where  $n$  is the index of refraction of the medium in which the lens is working, and  $\theta$  is the half-angle of the maximum cone of light that can enter or exit the lens



The numerical aperture with respect to a point  $P$  depends on the half-angle  $\theta$  of the maximum cone of light that can enter or exit the lens.

A numerical aperture is a dimensionless number that characterizes the range of angles the system can accept or emit light. It is the acceptance cone of an objective, and hence its light gathering ability and resolution. NA numerical aperture equal to

$$NA = n \sin \theta$$

where  $n$  is the index of refraction of the medium in which the lens is working, and  $\theta$  is the half-angle of the maximum cone of light that can enter or exit the lens.

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## **Magnification:**

**Magnification** is the process of enlarging something only in appearance, not in physical size

This enlargement is quantified by a calculated number also called "magnification"

A microscope, which makes a small object appear as a much larger object at a comfortable distance for viewing

A microscope is similar in layout to a telescope except that the object being viewed is close to the objective, which is usually much smaller than the eyepiece

Magnification is the process of enlarging something only in appearance and not in physical size. A calculated number quantifies this enlargement also called magnification. A microscope makes a small object appear a much larger object at a comfortable distance for viewing. A microscope is similar in layout to a telescope except that the object being viewed is close to the objective, which is usually much smaller than the eyepiece that the all the difference between microscope and telescope.

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## Condensers:

Condensers could be defined as a series of lenses for the illumination of the object under examination.

Several methods are employed for illustrating the object under examination: In Microbiology we have two methods commonly employed,

A) Illumination by transmitted light

The condensers could be defined as a series of lenses for the illumination of the object under examination. Several methods are employed for illustrating the object under examination in microbiology. We commonly employ two methods: illumination by transmitted light and darkfield illumination.

(Refer Slide Time: 19:35)

## Bright-field Microscopy:

The ordinary microscope is called as a bright field microscope. **It forms dark image against bright background**

The useful magnification of Light microscope is limited by its resolving power

The resolving power is limited by wavelength of illuminating beam

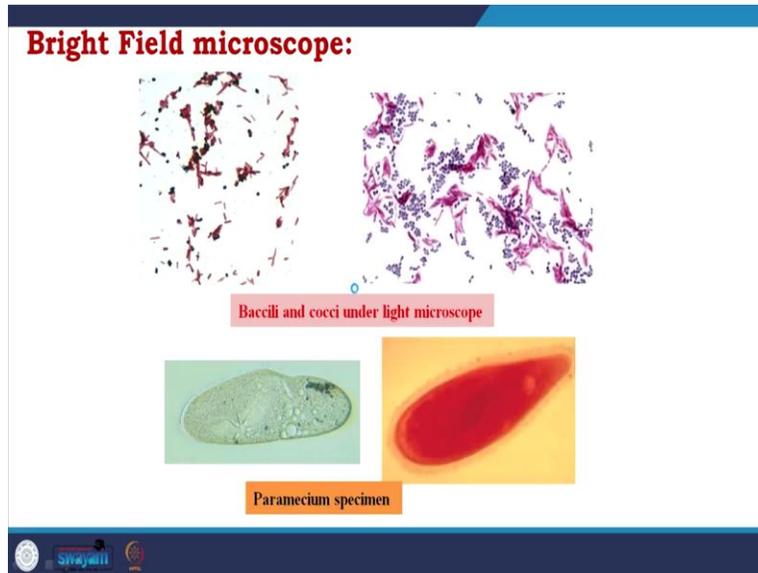
Resolution is determined by certain physical parameters like wave length of light and light generating power of the objective & condenser lens

Higher N.A Better light generation Better Resolution Shorter the Wavelength Better Resolution.

The ordinary microscope is called a bright field microscope. It forms the dark image against the bright background. The useful magnification of the light microscope is limited by its resolving power. The resolving power is limited by the wavelength of the illuminating beam. Resolution is determined by physical parameters like the wavelength and light-generating power of the objective and condenser lens.

Higher numerical aperture causes better light generation and resolution, whereas the shorter the wavelength, the better the resolution.

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Here, Baccili and Cocci are shown under a Bright field microscope where the background is bright, and the images' picture is dark. This is a Paramecium specimen again in a bright field common microscope.

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## Dark field microscope:

A bright-field microscope can be adapted as a dark-field microscope by **adding a special disc** called a stop to the condenser

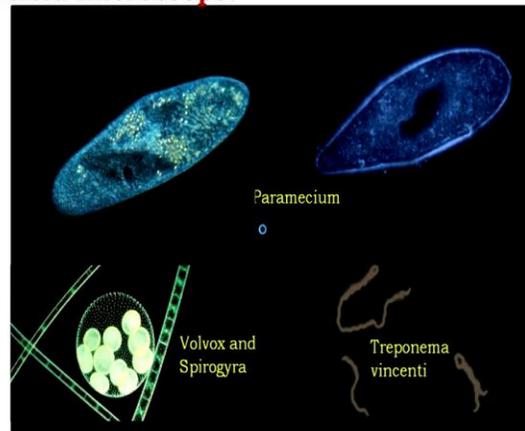
The stop blocks all light from entering the objective lens except peripheral light that is reflected off the sides of the specimen itself

The resulting image is a brightly illuminated specimens surrounded by a dark (black) field

The darkfield microscope creates an image with the background dark, and the picture is illuminated. So Bright field microscope can be adapted as a dark field microscope by adding a special disc called a stop block to the condenser. The stop blocks all light from entering the objective lens except the peripheral light reflected off the side of the specimen itself. The resulting images brightly illuminated specimens surrounded by a dark field.

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## Dark field microscope:



So these are the images created from the dark field microscope. You see, the background is dark, and we see Paramecium, wall box spirogyra, treponema vincenti, all of them are bright, and the background is dark that, is called dark field microscope.

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## Different Types of Microscopes:

One grouping is based on what interacts with the sample to generate the image:

- **Light or photons** (optical microscopes)

- **Electrons** (electron microscopes)

- **Probe** (scanning probe microscopes).



• Whether they analyze the sample via:

- **Scanning point** (confocal optical microscopes, scanning electron microscopes and scanning probe microscopes)

- **Analyze the sample all at once** (wide field optical microscope and transmission electron microscopes).



There are different microscopes. One could group based on what interacts with the sample to generate the image. So, when they interact, lights or photons are called optical microscopes, light microscopes. When it is the electron, it is called an electron microscope. When they have a probe, they have to scan, they have a width x, y, and then there will be a z-axis. So you get to know the depth. So you use a scanning probe that is called a scanning probe microscope.

And you could also group whether they analyze the sample as scanning point like a confocal optical microscope, scanning electron microscope, and scanning probe microscope. Analyze the sample all at once wide-field optical microscope and transmission electron microscope.

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## Electron Microscope:

Electron microscope was invented because of drawbacks of light microscope

Lights passing through slits get diffracted

The convex lens collects the diffracted light

After collection it produce image of the slit on the screen



Now we are coming to the electron microscope. The electron microscope was invented because of the drawback of the light microscope already discussed. The drawback is it is about resolving power. Light passing through slits gets diffracted, the convex lens collects the diffracted light. After collection, it produces an image of the slit on the screen.

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## Electron Microscope:

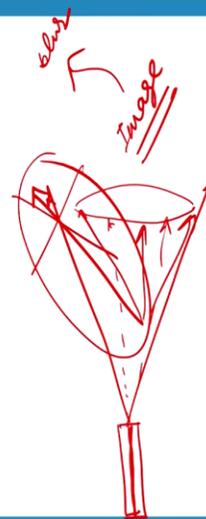
Edge of the slit used to diffract the light

To see the edge clearly as an image the lenses must collect the diffracted light

With the width of the slit decreased the diffraction increases

Now the objection of the ~~lense~~ failed to collect the diffracted pattern

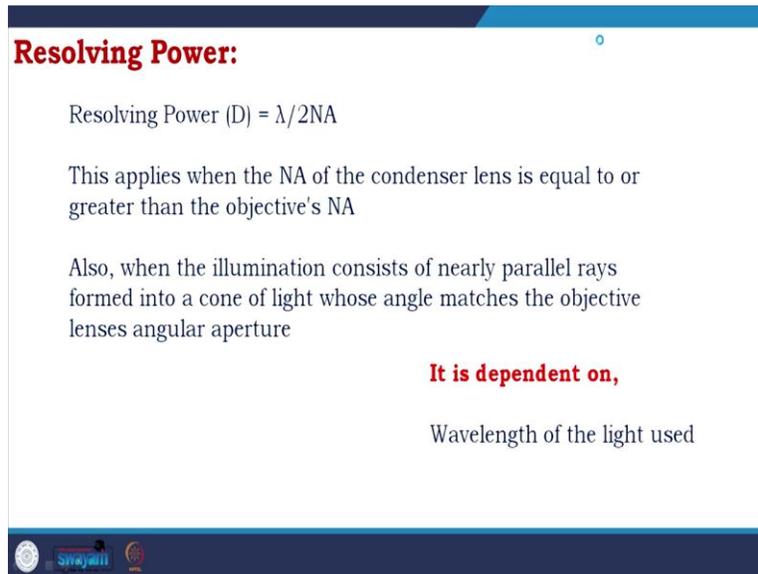
As a result blur images would be developed



The edge of the slit is used to diffract the light. The lenses must collect the diffracted light to see the edge clearly as an image. So you have the lens, and the lens have to collect all the refracted light. If the light passes through here, this is not collected. So when it creates the image, the image will be, blurred because it is losing the information here. With the width of the slit decreasing, the diffraction would be increased.

Now the objection of the lens fails to collect the diffracted pattern. It cannot collect all the diffraction. As a result, blur images would be developed. So the blurred image is created when the lens collects all the diffracted rays. When the slit is reduced, the reduced slit increases diffraction, unable to collect. So, information missing resulting in blur image that is the problem.

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**Resolving Power:**

Resolving Power (D) =  $\lambda/2NA$

This applies when the NA of the condenser lens is equal to or greater than the objective's NA

Also, when the illumination consists of nearly parallel rays formed into a cone of light whose angle matches the objective lenses angular aperture

**It is dependent on,**

Wavelength of the light used

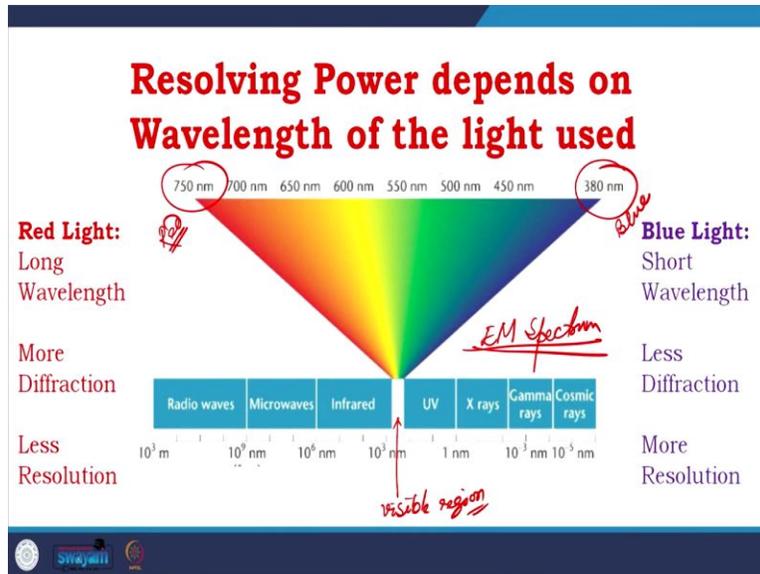
Resolving power

$$D = \lambda/2NA$$

NA is the numerical aperture, this applies when NA of the condenser lens is equal to or greater than the objectives NA. Also, the illumination consists of nearly parallel rays formed into a cone of light whose angle matches the objective lens angular aperture.

So, the resolving power quality depends on the wavelength of the light

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The resolving power depends on the wavelength of the light used. Now, if you see this is the electromagnetic spectrum. Here it is from the radio waves to the Cosmic rays where the wavelength is decreased. These are the visible region where we could see in the normal eye if you look at these. You will see that red has the highest wavelength and blue have the lowest wavelength. So when the wavelength is long more diffraction and less resolution.

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**de Broglie Equation:** Louis Victor Pierre Raymond de Broglie, was a French physicist and aristocrat who made groundbreaking contributions to quantum theory. In his 1924 PhD thesis, he postulated the wave nature of electrons and suggested that all matter has wave properties.



The de Broglie equation is an equation used to describe the wave properties of matter, specifically, the wave nature of the electron:

$\lambda = h/mv$ ,  
 where  $\lambda$  is wavelength,  $h$  is Planck's constant,  $m$  is the mass of a particle, moving at a velocity  $v$ , de Broglie suggested that particles can exhibit properties of waves.

The de Broglie hypothesis was verified when matter waves were observed in George Paget Thomson's cathode ray diffraction experiment and the Davisson-Germer experiment, which specifically applied to electrons.

I hope you all know about the De Broglie, Louis Victor Pierre Raymond de Broglie. He was a French physicist and aristocrat who made a groundbreaking contribution to Quantum theory. And when I say groundbreaking contribution, I would be surprised to know that this contribution was made while doing his doctoral studies. In his 1924 Ph.D. thesis, he postulated the wave nature of electrons and suggested that all matter has wave properties.

The De Broglie equation is used to describe the wave properties of matter, specifically the wave nature of electron, which is

$$\lambda = h / mv$$

$h$  is the Planck's constant  $m$  is the mass and  $v$  is the velocity.

De Broglie suggested that particles can exhibit the properties of a wave. The de Broglie hypothesis was verified when the George Paget Thomson cathode ray diffraction observed matter waves.

Cathode ray diffraction is the kind of the birth of electron and their existence and talking about that. The Davisson-Germer experiment specifically applied electrons, and since then, the De Broglie equation has been shown to apply to elementary particles, neutral atoms, and molecules.

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$$\lambda = h / mv$$

Wavelength is proportional to the mass

Mass of electron is  $9.10938356 \times 10^{-31}$  kilograms

Like electron, proton or neutron a photon is not a universal/unique particle

It's properties are dependent on the source

The **mass** of the **photon** from a Helium Neon Laser of 1.15 microns is equal to  $4.71 \text{ E-}38$  kilograms

It is easy to comment that what ever source be the origination of photon it's mass is much much less than electron

**So, wavelength of electron is much much less compared to photon**

*Mass photon is much less than electron*

$$\lambda = h / mv$$

the wavelength is proportional to mass. The mass of an electron  $9.1 \times 10^{-31}$ . Like electron, proton or neutron a photon is not a universal/unique particle. So it is dependent on the properties of the source. The mass of a photon from a Helium-neon laser of 1.15. Micron is equal to  $4.71 \text{ E}^{-38}$  kilograms. This is much, much lower than the mass of the electron. You could say photon has zero mass. It is easy to comment that whatever source be the origination of photon, its mass is much, much less than the electron. So, the electron's wavelength is much, much less than the photon.

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## **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

Electron microscopes use shaped magnetic fields to form electron optical lens systems that 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

An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination instead of a light or photon in a light microscope. The wavelength of an electron can be up to 100,000 times shorter than that of visible light photons. The electron microscope has a higher resolving power than a light microscope and can reveal the structure of smaller objects.

The electron microscope used shaped magnetic fields to form an electron optical lens system 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, a biopsy sample, metals, and crystals.

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## Electron Microscope: The Journey

In 1926, Hans Busch developed the electromagnetic lens

According to Dennis Gabor, the physicist Leó Szilárd tried in 1928 to convince him to build an electron microscope, for which he had filed a patent

The first prototype electron microscope, capable of four-hundred-power magnification, was developed in 1931 by the physicist Ernst Ruska and the electrical engineer Max Knoll

The apparatus was the first practical demonstration of the principles of electron microscopy

In May of the same year, Reinhold Rudenberg, the scientific director of Siemens, obtained a patent for an electron microscope

In 1926 Hans Busch developed the first electromagnetic lens started the journey. According to Dennis Gabor, the physicist Leo Szilard tried in 1928 to convince him to build an electron microscope for which he had filed a patent.

The first prototype electron microscope capable of 400 power magnification, 400 was developed in 1931 by the physicist on Ernst Ruska and electrical engineer Max Knoll. The Apparatus was the first practical demonstration of the principle of electron microscope. In May of the same year Reinhold Rudenberg, the scientific director of Siemens obtained pattern for an electron microscope. Siemens have a huge history as an industry. Have a huge history of connect their connection to the research on the electron microscope.

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## Electron Microscope: The Journey

In 1932, Ernst Lubcke of Siemens & Halske built and obtained images from a prototype electron microscope, applying the concepts described in Rudenberg's patent

In the following year, 1933, Ruska built the first electron microscope that exceeded the resolution attainable with an optical (light) microscope

Four years later, in 1937, Siemens financed the work of Ernst Ruska and Bodo von Borries, and employed Helmut Ruska, Ernst's brother, to develop applications for the microscope, especially with biological specimens

Also in 1937, Manfred von Ardenne pioneered the scanning electron microscope.

In 1932 Ernst Lubeke of Siemens and Halske built and obtained images from a prototype electron microscope applying the concept described in Rudenberg's pattern. In the following year, 1933, Ruska built the first electron microscope that exceeded the resolution attainable within an optical microscope.

Four years later, in 1937, Siemens again financed the work of Ernst Ruska and Bodo von Borries and employed Helmut Ruska and Ernst brothers to develop an application for the microscope, especially with the biological specimens.

Also, in 1937, Manfred Von Ardennen pioneered the scanning electron microscope.

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## Electron Microscope: The Journey

Siemens produced the first commercial electron microscope in 1938

The first North American electron microscope was constructed in 1938, at the University of Toronto, by Eli Franklin Burton and students Cecil Hall, James Hillier, and Albert Prebus

Siemens produced a transmission electron microscope (TEM) in 1939

Although current transmission electron microscopes are capable of two million-power magnification, as scientific instruments, they remain based upon Ruska's prototype

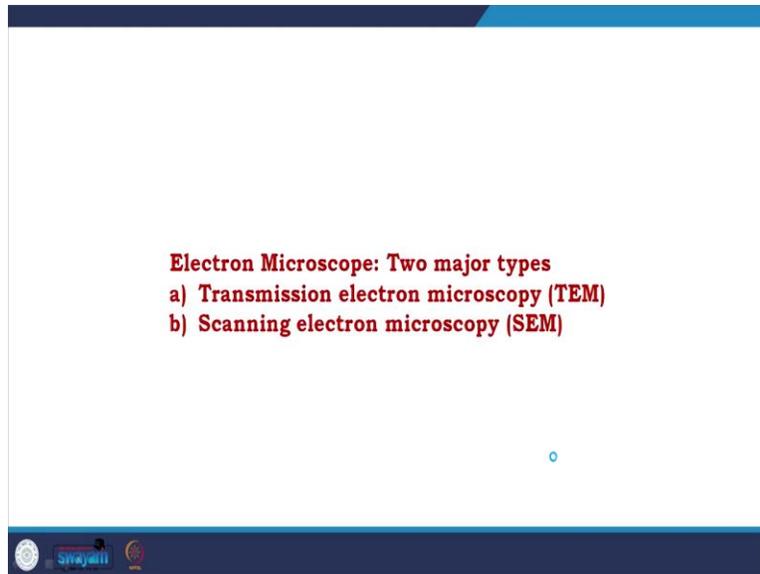
The Nobel Prize in Physics 1986 was divided, one half awarded to **Ernst Ruska** "for his fundamental work in electron optics, and for the design of the first electron microscope", the other half jointly to Gerd Binnig and Heinrich Rohrer "for their design of the scanning tunneling microscope"



Siemens produced the first commercial electron microscope in 1938. The first North American electron microscope was constructed in 1938 at the University of Toronto by Eli Franklin Burton and students like Cecil Hall, James Hillier, and Albert Priebus. Siemens produced a transmission electron microscope that came in 1939. Although current transmission electron microscopes are capable of two million power magnification, as a scientific instrument, they remain based upon Ruska's prototype.

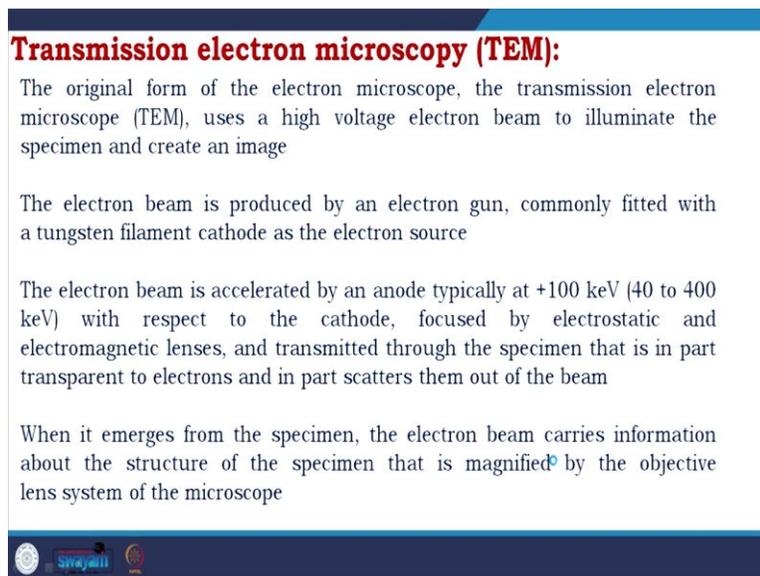
The Nobel Prize in physics 1986 was divided and one half awarded to Ernst Ruska for his fundamental work in electron optics. And also, for the design of the first electron microscope, the other half jointly to Gerd Binning and Henrich Rohrer for their design of the scanning tunneling microscope.

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So nowadays, there are many types of microscopes. They could be divided into two major types, like the transmission electron microscope (TEM) and scanning electron microscope (SEM).

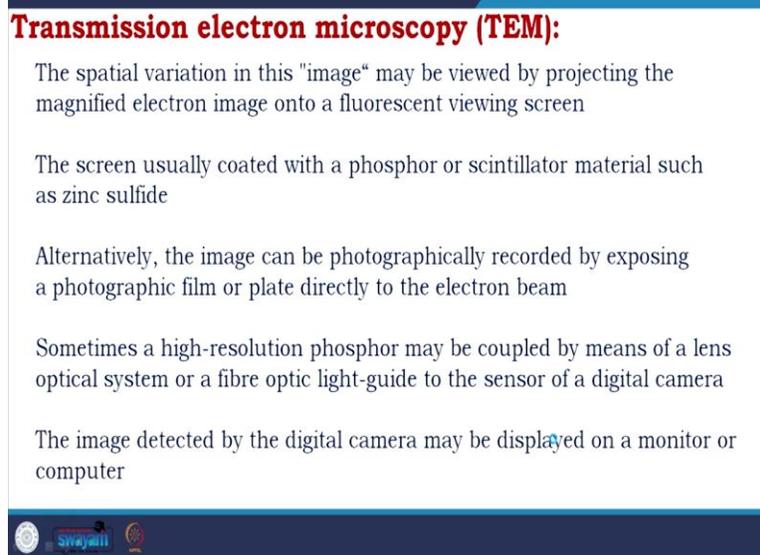
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Transmission Electron Microscope is the original form of electron microscope, the transmission electron microscope uses a high-voltage electron beam to illuminate the specimen and create an image. An electron gun produces the electron beam, commonly fitted with the tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at 100 kilovolt ranging from 40 to 400 kilovolt with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the partially transparent specimen to electron and in part scatters them out of the beam. When it emerges from the specimen, the electron beam

carries information about the specimen's structure that is magnified by the objective lens system of the microscope. So, the setup is the same as we discussed in the light microscope. Only the source is changed, and the compatible setup is changed to provide the creation and accommodation.

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**Transmission electron microscopy (TEM):**

The spatial variation in this "image" may be viewed by projecting the magnified electron image onto a fluorescent viewing screen

The screen usually coated with a phosphor or scintillator material such as zinc sulfide

Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam

Sometimes 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 spatial variation in this image may be viewed by projecting the magnified electron image into a frozen viewing screen. The screen is usually coated with phosphor or scintillator material such as zinc sulfide. Alternatively, the image can be photographically recorded using a photographic film or plate directly to the electron beam. So it could be coated with phosphor or scintillator material, or it could also be taken in a photographic film or plate.

Sometimes, a high-resolution phosphor may be coupled through a lens optical system or a fiber optic light guide to the digital camera's sensor. That is the modernized first. The image detected by the digital camera may be displayed on a monitor or a computer.

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### **Transmission electron microscopy (TEM):**

The resolution of TEMs is limited primarily by spherical aberration

This could be solved by using a new generation of hardware correctors which can reduce spherical aberration to increase the resolution in high-resolution transmission electron microscopy (HRTEM) to below 0.5 angstrom (50 picometers), enabling magnifications above 50 million times

The ability of HRTEM to determine the positions of atoms within materials is useful 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



The resolution of TEM's limited primary by Spherical aberration you remember Spherical aberration as you are talking about when it is diffracted the lens is unable to collect it, so you get blur image, so, your resolution has to be cut off. So, that is the cause of the resolution limit in the TEM. This could be solved by using a new generation of hardware characters that can reduce spherical aberration to increase the resolution in high-resolution transmission electron microscopy, called HRTEM High-Resolution Transmission Electron Microscopy to below 0.5 angstroms enabling magnification about 50 million times.

The ability of a high-resolution transmission electron microscope (HRTEM) to determine the positions of atoms within the material is useful for nanotechnologies research and development. Transmission electron microscopes are often used in electron diffraction mode. The advantage of electron diffraction over x-ray crystallography at that the specimen need not be a single crystal or even a polycrystalline powder. So, you do not have to face all we faced there in x-ray crystallography here.

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## Transmission electron microscopy (TEM):

Also 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

One major disadvantage of the transmission electron microscope is the need for extremely thin sections of the specimens, typically about 100 nanometers

Creating these thin sections for biological and materials specimens is technically very challenging

Biological tissue specimens are 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 staining with heavy atom labels in order to achieve the required image contrast



Also, The Fourier transform reconstructions of the object magnified structure occur physically, thus avoiding the need to solve the problem faced by the program after obtaining their x-ray diffraction pattern. One major disadvantage of the transmission electron microscope is the need for extremely thin specimen sectioning, typically about 100 nanometers.

Creating this thin section for biological and material specimens is technically very challenging. Biological tissue specimens are 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 staining with heavy atom levels to achieve the required image contrast.

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## Scanning Electron Microscope (SEM):

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image

The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition

### The main SEM components include:

- Source of electrons
- Column down which electrons travel with electromagnetic lenses
- Electron detector
- Sample chamber
- Computer and display to view the images



Now you are coming to the scanning electron microscope. This is the picture of the scanning electron microscope. A scanning electron microscope scans the focus electron beam over a surface to create an image. The electrons in the beam interact with the sample producing various signals that can be used to obtain information about the surface topography and composition. So it is talking about the surface, its width and all.

The main scanning electron microscope components include the source of electrons necessary for all electron microscopes. Column down which electron travels, electromagnetic lenses, electron detector, sample chamber and computer and display the images.

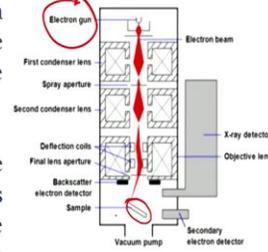
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## Scanning Electron Microscope:

Electrons are produced at the top of the column, accelerated down and passed through a combination of lenses and apertures to produce a focused beam of electrons which hits the surface of the sample

The sample is mounted on a stage in the chamber area and, unless the microscope is designed to operate at low vacuums, both the column and the chamber are evacuated by a combination of pumps

The level of the vacuum will depend on the design of the microscope



This is a schematic diagram of a scanning electron microscope. This is the electron gun. This is the source of the electron. Electrons are produced at the top of the column accelerated down and passed through a combination of lenses and apertures to produce a focused beam of electrons that hits the sample's surface. So it comes through, and here is the sample. So it is produced by the electron gun and then comes through the condenser lens and apertures, and all in it concentrate and hit the sample.

The sample is mounted on a stage in the chamber area. Unless the microscope is designed to operate at low vacuums, both the column and the chambers are evacuated by a combination of pumps, so high vacuum. So the vacuum levels depend on the microscope's design, but generally, a high vacuum is used.

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## Scanning Electron Microscope:

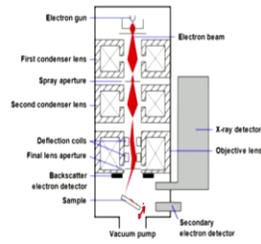
The position of the electron beam on the sample is controlled by scan coils situated above the objective lens

These coils allow the beam to be scanned over the surface of the sample

This beam rastering or scanning, as the name of the microscope suggests, enables information about a defined area on the sample to be collected

As a result of the electron-sample interaction, a number of signals are produced

These signals are then detected by appropriate detectors.



The position of the electron beam on the sample is controlled by the scan coil situated above the objective lens. So when you see here, we will see the coils present here. This is the objective lens. These coils allowed the beam to be scanned over the sample's surface. So these coils allowed the beam to be scanned over the surface. Rather than hitting it, scan the sample. As the microscope's name suggests, this beam restoring of scanning enables information about a defined area on the sample to be collected.

So it will not only take the surface. It also takes the width. As a result of the electron sample interaction, many signals are produced. Appropriate detectors then detect these signals.

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## Scanning Electron Microscope:

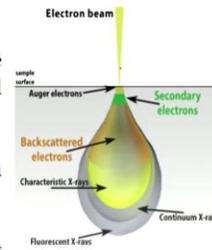
The scanning electron microscope (SEM) produces images by scanning the sample with a high-energy beam of electrons

As the electrons interact with the sample, they produce secondary electrons, backscattered electrons, and characteristic X-rays

These signals are collected by one or more detectors to form images which are then displayed on the computer screen

When the electron beam hits the surface of the sample, it penetrates the sample to a depth of a few microns, depending on the accelerating voltage and the density of the sample

Many signals, like secondary electrons and X-rays, are produced as a result of this interaction inside the sample



The scanning electron microscope produces images by scanning the sample with a high-energy beam of electrons. This high-energy electron comes and hits the surface. When it hits the surface, there are different effects like Fluorescent x-rays, continuum x-rays, characteristic x-rays, backscattered electrons, Auger electron, and secondary electron

One or more detectors collect these signals to form images displayed on the computer screen. When the electron beam hits the sample's surface, it penetrates the sample to a depth of a few microns, depending on the accelerating voltage and the density of the sample.

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## Scanning Electron Microscope:

The maximum resolution obtained in an SEM depends on multiple factors:

- the electron spot size
- interaction volume of the electron beam with the sample

While it cannot provide atomic resolution, some SEMs can achieve resolution below 1 nm

Typically, modern full-sized SEMs provide resolution between 1-20 nm whereas desktop systems can provide a resolution of 20 nm or more

The maximum resolution obtained by an electron microscope depends on multiple factors, like the electron spot size and interaction volume of the electron beam with the sample. While it cannot provide atomic resolution, some scanning electron microscopes can achieve resolution below 1 nanometer. Typically modern full-size SEMs provide resolution between 1 to 20 nanometers, whereas desktop systems can provide a resolution of 20 nanometers or more.

So what we did in this class, we started our topic of cryo-electron microscopy, and when we started cryo-electron microscope, this gave us a lot of basic questions. To answer them, we go to the concept of the microscope to know about what is light microscope? And how it started with the concept of observation. Observation with the limitation of the eye then eye limitation would be complemented with magnification lens being the simplest microscope.

Then comes the concept of objective lens and eyepiece lens that bring the concept of the compound microscope. The combination of series of object lens and eyepiece lens brings you the modern microscope. But in the modern optical microscope, there is a limit. So it gives us an idea that if we could replace Photon by electron.