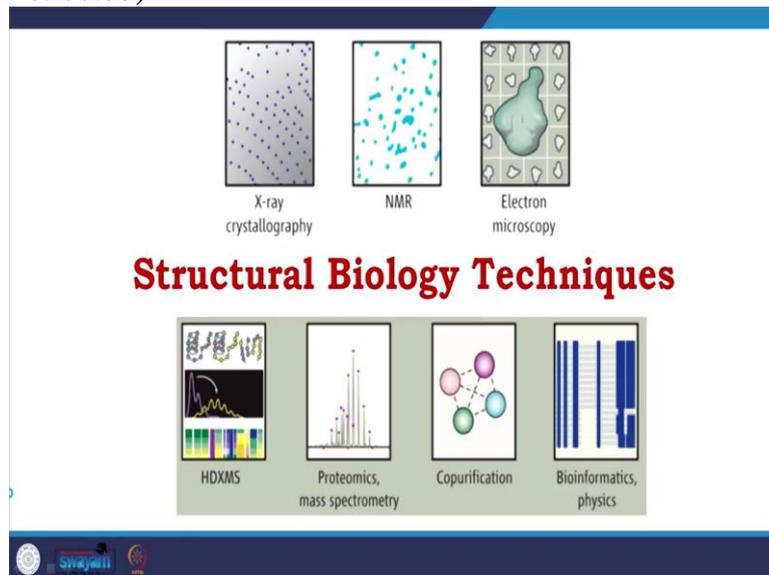**Structural Biology**
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Indian Institute of Technology – Roorkee

Lecture – 19
X-Ray Crystallography: Instrumentation in X-ray Crystallography

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Good day everyone. Welcome again to the course of structural biology. And as you know, we are going through structural biology techniques continuing with X-ray crystallography. We will learn something new from the basic subject, which is the instrumentation of X-ray crystallography.
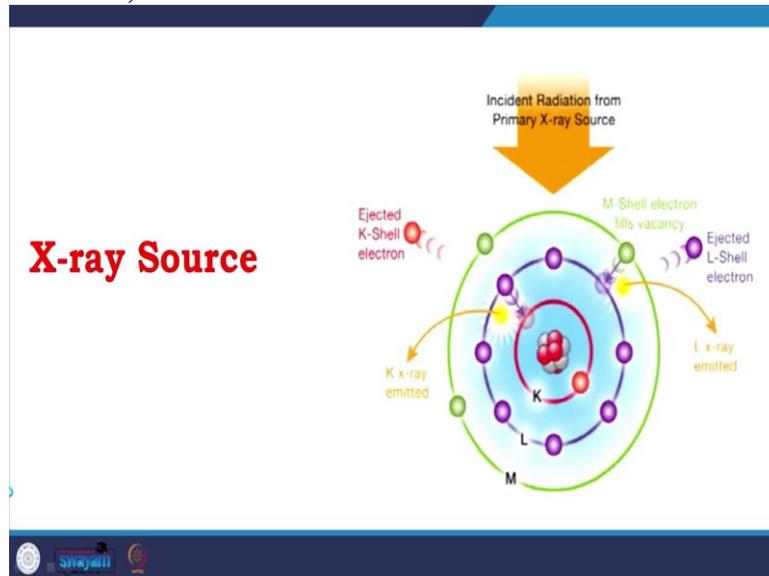
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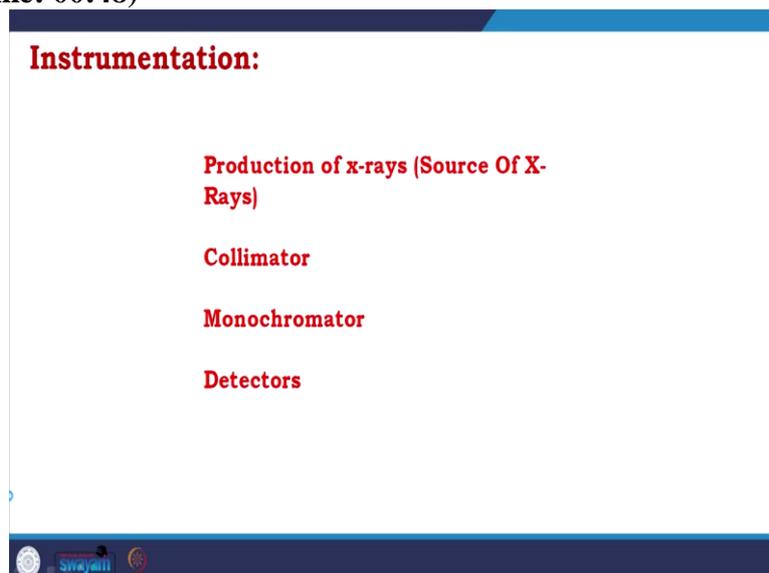
Majorly, the instrumentation is categorized as part of the production of X-rays, which is the source of X-rays. Then I will talk about collimators, something which is essential and helps

focus on monochromators. They also play a very interesting and critical role at the end detector. So, our instrumentation part is majorly categorized in these four subheadings. But when I talk about at the end, I will talk about where these guys are and how they are composed into a unit.

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Let us start with the X-ray source because it is X-ray crystallography. So, the source is the preliminary requirement Crookes tube, also called cold cathode tube, is not important anymore, but they are historically important; they are the X-ray source used in the early 1900s. When the initial X-ray crystallography experiments are used, these tubes generated the electrons needed to produce X-rays by ionizing the residual air in the tube.

They consisted of a glass bulb with around 10^{-6} to $5*10^{-8}$ atmospheric pressure of the air, and you could see the tube here. This is the construction of the tube. They had an aluminum

cathode plate at one end of the tube and a platinum anode target at the other end. So, this is the construction. Crookes tubes were unreliable, they used to be used at the initial stages, but then they did not continue because they were not reliable.

With time passed of their usage, the residual air would be absorbed by the tube walls, reducing the pressure. So, when the machine would be used multiple times becoming older, then the residual air would be absorbed by the walls of the tube. So, the pressure would be reduced. And this reduced pressure would increase the voltage across the tube that generates harder X-rays, and eventually, with time, the tube stopped working. So that is why it was not a good system to continue with.
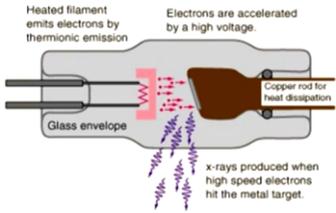
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Sealed tubes: We talked about sealed tubes, so I am not going into the entire detail. They are one composition where the entire X-ray generation system is sealed into 1 unit, thus eliminating the need for vacuum pumps, motors to spin the target extra. So, it is one compost unit with a heated filament that emits electrons by thermionic emission. The electrons are accelerated by high voltage. A copper rod for heat dissipation and X-rays produce when the high-speed electron hits the metal target.

But still, these are also not the best thing to use because one is overheating and then unable to produce high energy. So spatially, you know in our interest, when we are using it for protein, we need high energy, and sealed tubes cannot do that. So, you do not get that resolution.

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X-Ray Source: Rotating Anode

Rotating Anodes: A rotating anode allow the electron beam sweep a larger area of the anode, thus redeeming the advantage of a higher intensity of emitted radiation, along with reduced damage to anode compared to its stationary state.

The focal spot temperature can reach 2,500°C during an exposure, and the anode assembly can reach 1,000 °C following a series of large exposures.

Typical anodes are a **tungsten-rhenium** target on a **molybdenum** core, backed with **graphite**.

The rhenium makes the tungsten more ductile and resistant to wear from the impact of the electron beams.

The molybdenum conducts heat from the target. The graphite provides thermal storage for the anode, and minimizes the rotating mass of the anode.

Rotating anode is what people are using. A rotating anode allows the electron beam to sweep a larger area of the anode, thus redeeming the advantage of a higher intensity of emitted radiation and reducing damage to the anode compared to its stationary state.

The focal spot temperature can reach 2500 degrees centigrade during exposure, and the anode assembly can reach 1000 degrees centigrade following a series of large exposures. Typical anodes are a tungsten-rhenium target on a molybdenum core backed with graphite. Why? Because the rhenium; makes the tungsten more ductile and resistant to wear from the impact of the electron beams, the molybdenum conducts heat from the target. The graphite provides thermal storage for the anode and minimizes the rotating mass of the anode. These rotating anodes are still used and are specially used very comfortably for home sources.

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X-Ray Source: Synchrotrons

Synchrotrons: Synchrotrons are large tubular rings under high vacuum in which fundamental particles zoom at velocities near the speed of light.

Bending magnet costs energy to deflect (change the momentum of) all those particles.

That energy gets returned to us in the form of intense electromagnetic radiation when the particles change direction (velocity).

A lot of this radiation is in the X-ray band, and we can use it as a remarkably powerful X-ray source.

Bending magnet beamlines at Brookhaven (NSLS) are 50-100x brighter than a home source (e.g. X12C, X9A).

At Argonne (APS), the bending magnet beamlines are at least 1000x brighter than a home source

Synchrotrons are large tubular rings under a high vacuum in which fundamental particles zoom at a velocity near the speed of light. A bending magnet costs energy to deflect (change the momentum) of all those particles. That energy gets returned to us in the form of intense electromagnetic radiation; when the particles change direction, a lot of this radiation is in the X-ray band, and we can use it as a remarkably powerful X-ray source. I said that synchrotron used to be the toy of physicists now it has become more and more useful for others, especially protein crystallographers are using it hugely. There are a few reasons for that one, as when they started, things were not even in control. And crystallographers do not know how to use it, but now with several years of national facilities in US, Europe, and Japan, Australia. Now, people understand a lot about bending magnets. So, they know from where the energy is coming, and they have developed windows from where they have quantified the amount of energy coming. So, this helps the crystallographers to use this facility more and more. And how synchrotron is even more useful besides what we say I talked about quick data collection.

So, remember, if I talked about the home source with a rotating anode, which we used to use, in 2010, 2015, it used to be taking 3 to 7 days for an entire data collection, which means one frame used to be around depending on the signal 10 to 30 minutes. In a synchrotron, it is around 5 to 10 seconds. That is something, but another important thing that makes crystallography more fruitful with synchrotron is that one of the biggest things in crystallography is to grow crystals.

Sometimes you grow a crystal, but the crystals are very small. It isn't easy to diffract them from a home source with that tiny crystal. And it in that case, when we are getting not at all diffraction or diffraction at 7, 8 angstrom you could very well improve it to 2 angstroms, 1.5 angstroms with the help of synchrotron also for the data which you are collecting in home, it the resolution in many cases improves.

So, these are the facilities which we get in a synchrotron. So, bending magnet beamlines in Brookhaven, Brookhaven National Laboratory BNL. The facility called NSLS is 50 to 100 x brighter than the home source.

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X-Ray Source: Wigglers

Wigglers and undulators make the beam do just that - wiggle up and down.

Since the velocity is changing, electromagnetic radiation is produced but these devices are designed to produce a lot more local deviation in the trajectory (before returning to it's original path) so wigglers and undulators act as X-ray sources much brighter even than bending magnets.

A typical undulator is engineered to extremely high tolerances, features superconducting magnets, and is a few meters long.

Wiggler beamlines at Brookhaven (e.g. X25) are about 10x brighter than bending magnet beamlines.

In APS undulator beamlines are 10-100x brighter than the bending magnets.
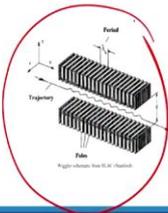
But this is great, but this is even better with the use of wigglers and undulators. These devices are designed to produce a lot more local deviation the trajectory before returning to its original path. So, what they do as it called the wiggler, it does the what means it we go up and down so, it makes more deviation wigglers and undulators act as X-ray sources much brighter even than the bending magnets.

So, a typical undulator is engineered to extremely high tolerances, features superconducting magnets, and is a few meters long. You could see here this is the schematics from slack Stanford, the synchrotron facility, so, wiggler beamline at Brookhaven is about 10 x brighter than the bending magnet beamline. So, it again changes, and when you see 10 x, 10 x is not less because it is 10 X on top of what the national synchrotron facilities were doing. And then, it comes to APS, and the undulator's beamlines are 10 to 100 x brighter than the bending magnet. So, it is great.

There are modern new facilities, modern sealed X-ray tubes, made up of ceramic, they are beryllium window visible, and you could see the anode type as copper written here and focus types are labeled. Besides that, there is a metal jet made up of gallium where they use liquid metal. So, remember the problem is when in the sealed tube it was continuously targeted, it was generating a lot of heat.

So, to solve it, we changed to rotation rotating anode so that it would not expose the same surface simultaneously. Now, they are coming up with even smarter technology. They use liquid gallium. So, each time the metal becomes bringing new gallium to the surface. So, the surface is changing continuously. So, these are the new techniques.

Collimator:

Collimator is a device that narrows a beam of particle or waves

This is to cause the directions of motion to become more aligned to a specific direction

In order to get a narrow, focused beam of x-rays, the x-rays generated by the target material are allowed to pass through a closely packed metal plates separated by a small gap.

The collimator absorbs all the x-rays except the narrow beam that passes between the gap.

The collimator is a device that narrows a beam of particles or waves. So, the beams generated by the X-ray source, collimator are narrowing them down. This is to cause the direction of motion to become more aligned to a specific direction so that they could hit the target in the X-ray diffractometer. So, you could see here that the beams are coming here. They are in a different direction and aligned here. This is the function of the collimator. To get a narrow-focused beam up X-rays, the X-rays generated by the target material are allowed to pass through a closely packed metal plate separated by a small gap. So, I showed you two metal plates, and instead of 2 metal plates, they use a series of metal plates with some gaps. So, this is what used so the closely packed metal plates. Small gaps separate them, and they channelize the beams. The collimator absorbs all the X-rays except the narrow beam that passes between the gaps, so only allowed ones are going and working. The rest of them are absorbed.
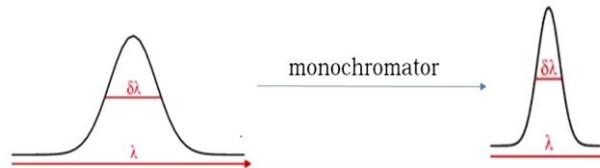
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Coming to monochromator: As you understand, the polychromatic rays are becoming monochromatic. A monochromator is an optical device that transmits a mechanically selectable narrow band of wavelengths chosen from a wider range of wavelengths available at the input. So, if you see it here, it is wider. It becomes narrower with the help of a monochromator. The name is from the Greek roots of mono. Mono is single, chroma is color, and ator is denoting agent. So, an agent who makes a single color is called a monochromator. Monochromator crystals partially polarize the unpolarized light.

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Monochromatization can be broadly divided into two classes. One interference filter and two crystal monochromators. Again, crystal monochromators can be divided into flat crystal monochromators and curved crystal monochromators.
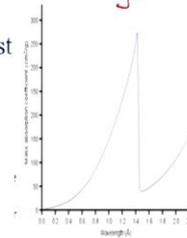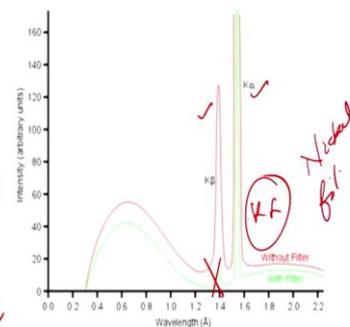
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Interface filters exploit the X-ray absorption edge of a particular element. Wavelength longer than the absorption edge. When I say longer than the absorption edge, it means just above the absorption edge, the absorption of the X-rays is considerably less. So, it takes the principle that at wavelengths longer than the absorption edge, which is just above the absorption edge, the absorption of X-rays is considerably less, and at the reverse at wavelengths shorter than the absorption edge is quite high. So, this is the scenario of nickel.

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The absorption edge of nickel-metal, which is 1.488 angstrom, lies between Kα, which is 1.542, and Kβ, which is 1.392 X-ray spectral line of copper. Copper is used specially for protein X-rays, and it is a very fortunate situation here that nickel its edge is between the Kα and Kβ. So, they could easily separate them. Hence, nickel foil of an appropriate thickness is used to reduce the copper Kβ X-rays.

When it says appropriate how you mean or how you understand which; is appropriate thickness, I will answer what an appropriate thickness is. So, if you see without filter, you have Kα and Kβ both, but it is nearly not there with filter Kβ. So, you only get Kα, which is the beauty of using a nickel filter.

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Choice of filter material:

The choice of filter depends on the anode material choosen in the X-ray tube

Anode	Cu	Co	Fe	Mo	Cr
Filter	Ni	Fe	Mn	Zr	V

The beam passes through the top filter which is nickel in this case.

Alternative metal foils can be set by rotation of the filter housing towards appropriate position

One position of the filter housing kept open for the case where no filter would be required

Choice of filter material, how we could choose the filter material, the choice of filter depends on the anode material chosen in the X-ray tube-like for copper. I have shown we use nickel. For cobalt, we use iron. For iron, we use manganese. For molybdenum, we use Zirconium. For chromium, we use vanadium. The beam passes through the top filter, which is nickel in this case. Alternative metal foils can be set by rotating the filter housing towards the appropriate position.

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Effect of Filter:

Target	Kα (Å)	β-filter	Thickness (µm)	Density (g/cc)	% Kα	% Kβ
Cr	2.291	V	11	6.00	58	3
Fe	1.937	Mn	11	7.43	59	3
Co	1.791	Fe	12	7.87	57	3
Cu	1.542	Ni	15	8.90	52	2
Mo	0.710	Zr	81	6.50	44	1

How to choose filter thickness?

$K_\alpha / K_\beta \rightarrow 25:1 \rightarrow 50:1$

So, this is the effect of the filter. You will see where the different metal chromium, iron, cobalt copper, and molybdenum are filtered using different other metals. And if you see the thickness in the case of chromium, it is 11. For iron, it is 11. In cobalt, it is 12. For copper, it is nickel which is 15, and in molybdenum, it is zirconium 81.

So, I have asked how to choose the filter thickness. If there is no rule, you have to do trial and error, and generally, the things come that if you get Kα by Kβ ratio within 25:1 to 50:1 in between, you choose that. So, the thickness is optimized based on the ratio up Kα, Kβ percentage available.
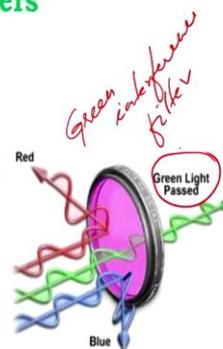
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Monochromator: Types : Interface Filters

X-ray beam may be partly monochromatized by insertion of a suitable filter.

A filter is a window of material that absorbs undesirable radiation but allows the radiation of required wavelength to pass.

Interference filters contain several optical layers deposited on a glass substrate or transparent quartz.

The specific performance characteristics of the filter are determined by the thickness of the optical layers.

Interference filters: X-ray beam may be partly monochromatized by insertion of the suitable filter. A filter is the window of material that absorbs undesirable radiation but allows the radiation of the required wavelength to pass. Interference filters contain several optical layers deposited on a glass substrate or transparent quartz. The specific performance characteristics

of the filter are determined by the thickness of the optical layers like here, and you see the interference filter. This is called a green interference filter. Because when the red is coming, it reflects blue, but green is allowed to pass.

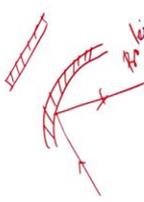So, as discussed, X-ray filters are used to reduce the unwanted white radiation from the X-ray source and eliminate the Kβ radiation. The major drawback of a filter is that the background radiation is still high, and transmitted radiation is still not monochromatic.

Crystal monochromator: Crystal monochromators are made up of suitable double crystalline material position in the X-ray beam. They help the angle of reflecting planes satisfy Bragg's equation for the beam's required wavelength split up into component wavelengths. Crystals used in monochromators are made up of sodium chloride, lithium fluoride, quartz, etc.

Pyrolytic graphite can also be used for broadband, and silicon could be used for narrowband. So, as I told you, there are 2 types of crystal monochromators: flat and straight and bend. The bend one is helping the circular light to deflect, and they are mostly working on protein.

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Advantage of monochromatic rays:

Help in improving resolution

Minimizing sample preparation

Improved signal to noise ratio

Analyze small samples

Multispotting on sample

Simplified data processing

So, what are the advantage of monochromatic rays? They help improve resolution, minimize sample preparation, improve the signal-to-noise ratio. They analyze small samples, and they also help multi-spotting on the samples. They simplify the data processing because you do not get the interference of the other one.
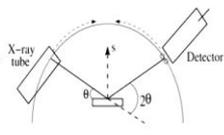
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X-ray detectors:

X-ray detectors are devices used to measure the flux, spatial distribution, spectrum, and other properties of X-rays.

Detectors can be divided into two major categories:

A) Imaging detectors, such as photographic plates and X-ray film (photographic film)
now mostly replaced by various digitizing devices like image plates or flat panel detectors)

B) dose measurement devices called counter methods.

Now, coming to X-ray detectors, X-ray detectors are devices used to measure X-rays' flux, spatial distributions spectrum, and other properties. Detectors can be divided into two major categories—imaging detectors such as photographic plates and X-ray film. The photographic

film we used before is mostly replaced by various digitizing devices like image plates or flat channel detectors and counter methods.
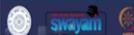
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X-ray detectors:

The x-ray intensities can be measured and recorded either by ,
1)Photographic methods

2)Counter methods
a) Geiger - Muller tube counter
b) Proportional counter
c) Scintillation detector
d) Semi conductor detectors

Both these types of methods depends upon ability of x-rays to ionize matter and differ only in the subsequent fate of electrons produced by the ionizing process.

The X-ray intensities can be measured and recorded by photographic, and counter methods. There are various devices under the counter method: Geiger-Muller tube counter, proportional counters, scintillation detector, and semiconductor detectors. Both this type of method depends upon the ability of the X-ray to ionize matter and differ only in the subsequent fate of the electron produced. So, how the electrons are ionized and what they are detecting how sensible that is the main factor here.

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Photographic Method:

To record the position and intensity of x-ray beam a plane or cylindrical film is used.

The film after exposing to x-ray is developed The blackening of the developed film is expressed in terms of density units D given by
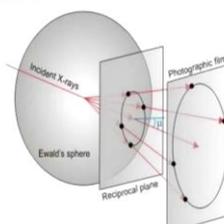**D = log I₀/I**, I₀-incident intensity I - Transmitted intensity
D - Total energy that causes blackening of the film
D is measured by densitometer
The photographic method is mainly used in diffraction studies since it reveals the entire diffraction pattern on a single film

Disadvantage : time consuming and uses exposure of several hours

The photographic method, you could see the incident rays coming, and under the Ewaid's sphere, which we will discuss later in the next class, there is a reciprocal plane and a photographic film is kept there. So, to record the position and intensity of the X-ray beam, a

plane or cylindrical film is used. The film, after exposing to X-rays, developed the blackening of the developed premise expressed in terms of density where

$D = \log I_0 / I$.

I_0 is the incident intensity, I is the transmitted intensity, and D is the total energy that causes the blackening of the film. So, it is the photo X-ray films you now see, when used for the patients to take their condition of bone or something in the hospital. A densitometer measures' D'. The photographic method is mainly used in diffraction studies since it refills the entire diffraction pattern on a single film.

It is time-consuming and uses exposure up to several hours, as you can understand. We have changed the spectrum now, and we have devices which does these things much with much speed and accuracy.
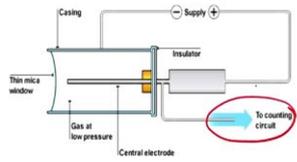
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Geiger-Muller tube counter: Geiger tube is filled with an inert gas like argon. Central wire anode is maintained at an 800 to 1500 volts positive potential. The electron is accelerated by the potential gradient and causes the ionization of many argon atoms. So, argon is inert, which is why side interference would happen. This results in the avalanche of electrons traveling towards the central anode, which is the target.

So, this is the setup. This is the center electrode, and here you will see the counting circuit. When the electron comes, they make the factor here towards detection.

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Counter Methods: B) Proportional counter:

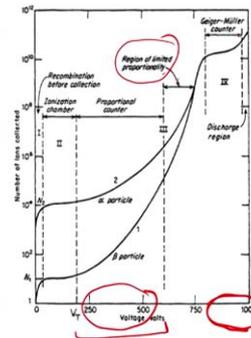Construction is similar to Geiger tube counter

Proportional counter is filled with heavier gas like xenon and krypton

Heavier gas is preferred because it is easily ionized

Operated at a voltage below the Geiger plateau

The dead time is very short (~ 0.2μs)

It can be used to count high rates without significant error.

Proportional counter: The setup is similar to the Geiger tube counter. The proportional counter is filled with heavier gas like xenon, and krypton, which are heavier than argon. They use heavier gases because they are easily ionized and operated below the Geiger plateau voltage. The dead time is very short, 0.2 μS, it can count high rates without significant error. So, if you see the voltage here around 250 to 500 whereas, in Geiger-Mueller, it is around 800 to about 1000, the voltage is less, and you will see how it is like producing the region of limited proportionality. This is if you compare you will see the difference.
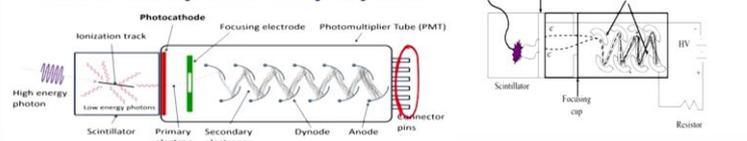
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Counter Methods: C) Scintillation detector

In a scintillation detector there is large sodium iodide crystal activated with a small amount of thallium

When x-ray is incident upon crystal, the pulses of visible light are emitted which can be detected by a photo multiplier tube

Useful for measuring x-ray of short wavelength

Crystals used in scintillation detectors include sodium iodide, anthracene, napthalene and p-terphenol

Scintillation detector: A large sodium iodide crystal is activated with a small amount of thallium in a scintillation detector. When an X-ray incident upon the crystal, the pulses of visible light are emitted, which can be detected by a photomultiplier tube, so useful for measuring X-ray of short wavelength crystal used in scintillation detector includes sodium iodide anthracene, naphthalene, and p-terphenol.

If you look at it here, they have the conductor pin, which you could install anywhere. This is the anode, and you have the scintillation that is hitting the cathode and these are the part of the photomultiplier tube that enhances the signal.

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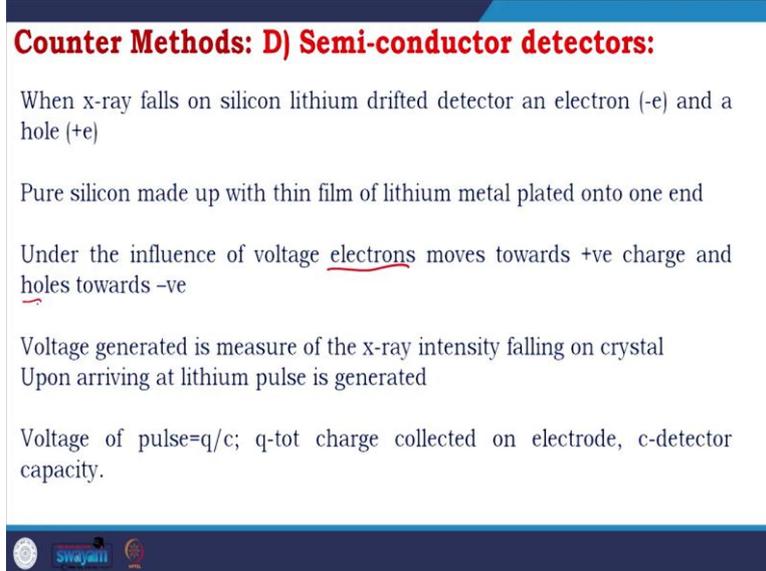
Semiconductor detector: An electron (-e) and hole (+e) when an X-ray falls on silicon lithium drifted detector. Pure silicone comprises a thin film of lithium metal plated onto one end. Under the influence of voltage, electrons move towards positive charge and holes towards negative. So, there is a difference between the electrons and the holes. Holes here work as a positive voltage generated to measure the X-ray intensity falling on crystal upon arriving at lithium pulse is generated.

The voltage of pulse = q / c.

q is the total energy collected on the electrode, and c is the detector capacity.

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X-ray Diffractometer:

A diffractometer is a measuring instrument for analyzing the structure of a material from the scattering (diffraction) pattern produced when a beam of radiation or particles (in our case X-rays) interacts with it.

A typical diffractometer consists of a source of radiation (X-ray source) to choose the wavelength

It needs a collimator to make the beam parallel

It contains a set up of sample holding

It needs a detector to detect the diffracted signal

How the whole thing is composed a diffractometer is the measuring instrument for analyzing the structure of a material from the scattering, which means diffracting pattern produced when a beam of radiation or particle interacts with it, you have a material you put the X-ray, and it makes a scattering pattern or diffraction pattern, if the pattern would help you to calculate the property of this material, then it is useful that is what happening in the diffractometer.

A typical diffractometer consists of a radiation X-ray source to choose the wavelength like when you were working with the protein and using copper. Copper is 1.54 Kα. If you think this is copper, you need two wavelengths. Why? We, Will, come when we are going to solve this problem initially next few classes, we are going to solve. So, when we take the first problem, we need a reference. So, instead of you having copper, you need another one.

So, you have to choose that right, it needs a collimator to make the beam parallel. We already know about collimator; we know how collimator works. So, the diffractometer needs a collimator to make the beam parallel. So, you need a nitrogen gas set up, and you need the crystal to set up in a balanced way in the goniometer so that you can automatically change the angle of data collection. So, all these are set up of the sample holding it needs a detector to detect that diffracted signal. We talked about those detectors.

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An X-ray diffractometer illuminates the samples of material with X-rays of known wavelength. A strip of X-ray film is mounted in a circular position around the sample. The undeviated central beam passes through a hole E cut in the film strip P, and the diffracted beam falls on the film at various points like d1, d2, d3, d4.

So, you change it so that this happens and intensities of the diffraction peaks are proportional to the fraction of the material in the mixture. So, you are getting it, and from the pattern, you could calculate which is what we will read in the next class.

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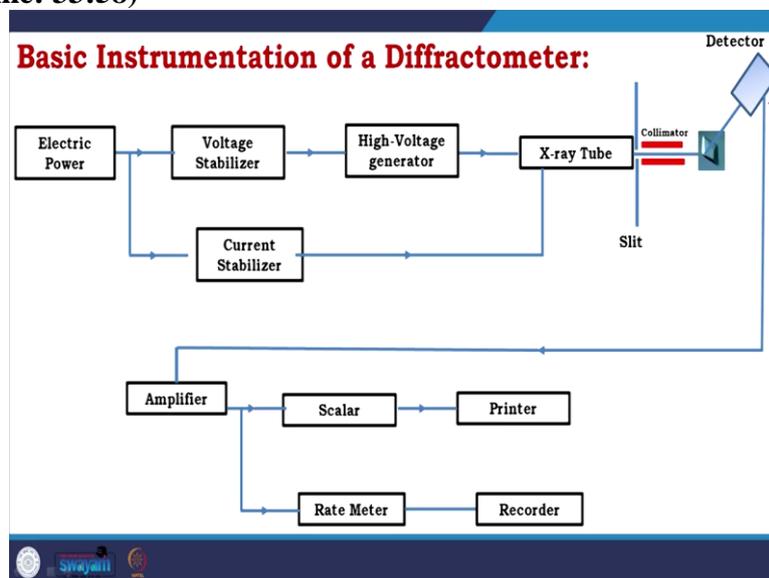
So, I have developed a simple instrumentation set up of the deflectometer you need the electric power, which go through voltage stabilizer and current stabilizer going to high voltage generator and coming to X-ray tube it might be X-ray tube it might be rotating anode it might be synchrotron it might be wiggler, or it might be undulator anything, it needs that

X-ray to provide. Then it goes through the slit and the collimator which we talked about the column it will align it and then hit the crystal and the detector is going to detect it.

That is the basic setup but in addition to that, now you have to get the signal. So, you have the amplifiers, scalar, printer, rate meter and recorder which are the secondary devices which will record the data.

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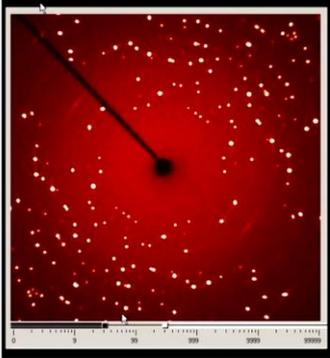
So, what next, we now would get the X-ray diffraction pattern from a single crystal and if you look at this image. This image happens to be a 360-degree rotation in phi collected on a CCD detector with all goniometer angles set to 0. Note that the X-ray diffraction pattern from a single crystal is a set of spots. So, you could see the set of spots and do you understand what is there if you look at you will get an idea of a periodicity which is what we need.

So, in the next class, we will talk about how this diffraction pattern used to be. In previous class we discuss about the ordering of the crystal when we were talking about crystallization that if there are translational or rotational degrees of distortion that affect the diffraction pattern. So, how the diffraction pattern will come? How we would use these to measure something.

And how we get to solve the protein structure would be our future discussion. Thank you very much. You guys are lovely audience and I hope that you understand from this class, if you have any queries, please let us know. I will try my best to answer. Thank you.