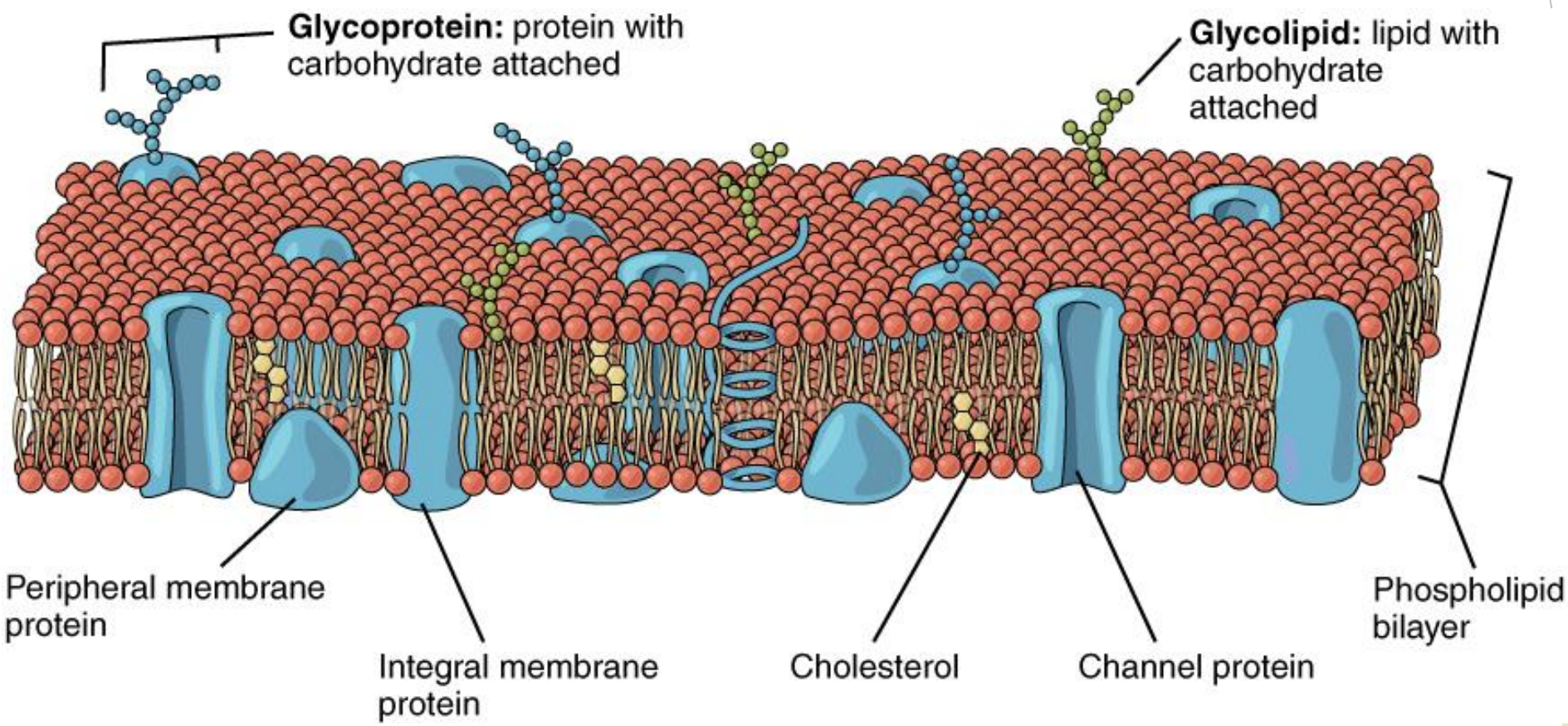
Methods to study membrane structure and properties

- The cell membrane is a biological membrane that separates the interior of all cells from the outside environment which protects the cell from its environment.
- The cell membrane consists of a lipid bilayer including cholesterols that lie between phospholipids to maintain their fluidity at various temperatures



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File:0303 Lipid Bilayer With Various Components.jpg - Wikimedia Commons

From traditional method such as:

- Electron Microscopy
- X-ray crystallography
- Fluorescence Microscopy
- Nuclear Magnetic Resonance Spectroscopy
- Mass spectroscopy
- To modern methods like Atomic Force Microscopy (AFM)
- Single molecular tracking
- Super - resolution fluorescence microscopy (SRFM)

OVERVIEW

- ***X- ray crystallography*:-** Use for determining the atomic and molecular structure of a crystal
- ***Fluorescence microscopy*:-** To observe the localization of molecules within cells ,and of cells within tissues
- ***Nuclear magnetic spectroscopy*:-** Use to obtain detailed information about the structure, dynamics, reaction state, and chemical environment of molecules
- ***Atomic force microscopy*:-** It use to study unfolding of protein, study surface frictional force, imagining of Biomolecules

Fluorescence Microscopy

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File:Olympus-BX61-fluorescence microscope.jpg - Wikimedia Commons

- Fluorescence microscopy discover by British Scientist Sir George G. Stokes
- When he observed that the mineral fluorspar emitted red light when it was illuminated by ultraviolet excitation .
- Early investigations in the 19th century showed that many specimens (including minerals, crystals, butter, chlorophyll) fluoresce with irradiated with UV light.
- However, it was not until 1930 use of fluorochrome was initiated in biological investigation.

- The technique of fluorescence microscopy has become an essential tool in biology and the biomedical sciences, as well as in materials science due to attributes that are not readily available in other contrast modes with traditional optical microscopy.
- The application of an array of fluorchrome has made it possible to identify cells and sub-microscopic cellular components with a high degree of specificity amid non-fluorescing material.
- In fact, the fluorescence microscope is capable of revealing the presence of a single molecule.
- Through the use of multiple fluorescence labelling, different probes can simultaneously identify several target molecules simultaneously.
- Although the fluorescence microscope cannot provide spatial resolution below the diffraction limit of specific specimen features, the detection of fluorescing molecules below such limits is readily achieved.

- The basic function of a fluorescence microscope is to irradiate the specimen with a desired and specific band of wavelengths, and then to separate the much weaker emitted fluorescence from the excitation light.
- In a properly configured microscope, only the emission light should reach the eye or detector so that the resulting fluorescent structures are superimposed with high contrast against a very dark (or black) background.
- The limits of detection are generally governed by the darkness of the background, and the excitation light is typically several hundred thousand to a million times brighter than the emitted fluorescence.

Principle

- Most cellular components are colourless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with dyes.
- Fluorescent dyes, also known as fluorophores or fluorochromes, are molecules that absorb excitation light at a given wavelength (generally UV), and after a short delay emit light at a longer wavelength.
- The delay between absorption and emission is negligible, generally on the order of nanoseconds.
- The emission light can then be filtered from the excitation light to reveal the location of the fluorophores.

X-ray crystallography

- X-ray crystallography is currently the most favoured technique for structure determination of proteins and biological macromolecules.
- The aim of *X*-ray crystallography is to obtain a three dimensional molecular structure from a crystal
- The method revealed the structure and function of many biological molecules, including vitamins, drugs, proteins, and nucleic acids, such as DNA.

Principle

- The crystalline atoms cause a beam of X-rays to diffract into many specific directions by measuring the angles and intensities of these diffracted beams,

- a crystallographer can produce a 3D picture of the density of electrons within the crystal.

Nuclear magnetic spectroscopy

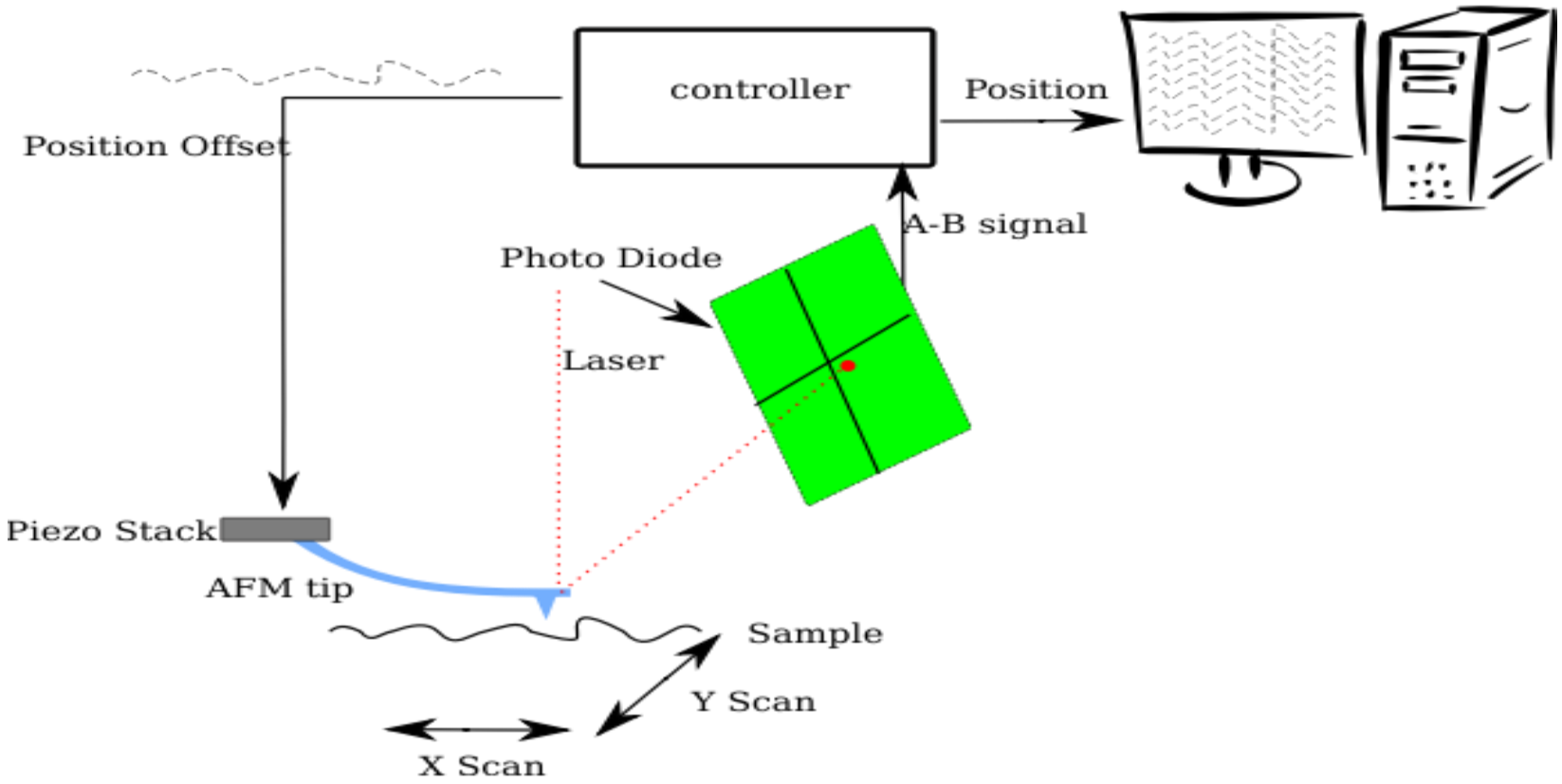
- Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei.
- It is a spectroscopy technique which is based on the absorption of electromagnetic radiation in the radio frequency region 4 to 900 MHz by nuclei of the atoms.
- Over the past fifty years, NMR has become the preeminent technique for determining the structure of organic compounds.
- Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected.

Principle

- The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).
- The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.
- The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

Atomic force microscopy (AFM)

- Atomic force microscopy (AFM) is a technique for analyzing the surface of a rigid material all the way down to the level of the atom
- AFM uses a mechanical probe to magnify surface features up to 100,000,000 times, and it produces 3-D images of the surface
- The technique is derived from a related technology, called scanning tunnelling microscopy (STM)
- The difference is that AFM does not require the sample to conduct electricity


controller
Position
Position Offset
A-B signal
Photo Diode
Laser
Piezo Stack
AFM tip
Sample
Y Scan
X Scan

Principle

- The Atomic Force Microscope works on the principle measuring intermolecular forces and sees atoms by using probed surfaces of the specimen in nanoscale.
- Its functioning is enabled by three of its major working principles that include Surface sensing, Detection, and Imaging
- The Atomic Force Microscope (AFM) performs surface sensing by using a cantilever.
- The cantilever has a sharp tip which scans over the sample surface, by forming an attractive force between the surface and the tip When it drawn Closer to sample surface.

- During the deflection of the cantilever away from the sample surface, there is a change in direction of reflection of the beam and a laser beam detects the aversion, by reflecting off a beam from the flat surface of the cantilever.
- Using a positive-sensitive photo diode (PSPD) It tracks these changes of deflection and change in direction of the reflected beam and records them.
- The Atomic Force Microscope (AFM) takes the image of the surface topography of the sample by force by scanning the cantilever over a section of interest.
- Depending on how raised or how low the surface of the sample is, it determines the deflection of the beam, which is monitored by the Positive-sensitive photo-diode (PSDP).
- The microscope has a feedback loop that controls the length of the cantilever tip just above the sample surface, therefore

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