Microscopy in biology

paper: Microscopy in Biology paper, In olden times, due to non availability of any kind of microscope, only macroscopic structures of an organism were studied. Later, owing to the invention of magnifying lenses, the world of microscopic dimensions was discovered.

It was found that a single cell can constitute an entire or?ganism as in Protozoa, or it can be of many cells that are grouped and differentiated into tissues and organs to form a multicellular organism. The development and refinement of microscopic techniques made it pos?sible to gain further knowledge of cellular structure. Observation of biological structures is difficult since cells are very small and are transparent to visible light. The majority of cell components are transparent, except for some pigments present in plant cells. They absorb light at certain wavelengths (coloured substances). The low light absorption of the living cell is caused largely by its high water content. The cell components show little contrast even after drying. To overcome this difficulty, we use dyes that selectively stain different cell components to produce contrast by light absorption.

Resolving Power: The purpose of any microscope is to make visible an object that normally can not be seen, or can not be seen clearly, by the naked eye. The property of a microscope is their ability to clearly distinguish separate parts of an image. This property of microscope is termed its resolving power. The resolving power of a microscope is the capacity to show distinct images of points which are very close together or the smallest separation at which we can distinguish two objects rather than one. Thus, for example, if two parts of an image are 0. 01 ? µm apart, they can be resolved as separate entities by an electron microscope, which has a resolving power of 0. 5 nm (0. 0005 ? µ.

m), but not by a light microscope, which has a resolving power of 0. 2 ? µm. The resolving power of a light microscope is approximately equal to one half the wavelength of light used to illuminate the object.

The resolving power depends upon the wavelength (lambda, ?) and numerical aperture (NA) of the objective lens. Limit of resolution is defined as the minimum distance between two points that allows for their discrimination as two separate points. Limit of resolution (r) = 0. 61 ? / NANA is the numerical aperture and it is equal to n x sin ?. Here, n is the refractive index of the medium and sin ? is the sine of the semi angle of aperture. The limit of resolution is inversely related to the resolving power, i.

e., the higher the resolving power, the smaller the limit of resolution. Since sin ? can not exceed 1, and the refractive index of most optical material does not exceed 1. 6, the maximal NA of lenses, using oil immersion, is about 1.

4. With these parameters it is easy to calculate the limit of resolution of the light microscope that can not exceed 170 nm (0. 17 ? µ. m) using monochromatic light of ?= 400 nm (violet).

With white light, the resolving power is about 250 nm (0. 25 ? µm). Since here the NA is limited, it is evident that the only way to increase the resolving power is to use shorter wavelengths. paper paper -X