Advantages: particular focal point and light coming

Advantages:· It provides high contrast imaging becauseit only provides us the idea of which region of the cell contain fluorescentdye and which is not.· High specificity since we tag thefluorescent dye with our interested cellular components.· Quantitative imaging and live cellimagingDisadvantage:· Photo bleaching ConfocalMicroscopy: Confocal microscopy isan optical microscopic technique for increased resolution and contrast. Thereconstruction of 3D structure from the obtained images can be done. Thespecial feature here is the pinhole, which only selects the light coming from aspecial focal point of the sample and blocks any other scattered lights thatare coming from the different planes of the specimen. It allows only particularpoint to be focused and only detect light or emission that is coming from thespecific point which eventually gives crystal clear images. The idea about thefluorescence is same as the fluorescence microscopy, the pinhole is placedwhich only allows the emission coming from the particular plane of the specimento pass and block any other excitation.

Concise and contrast image from theparticular location can be seen. Pinhole provides the optical sectioning whichallows blocking the emission light from out of plane regions of the sample andonly select one particular focal point and light coming from that point passesthrough the pinhole to the detector. WorkingPrinciple: When the laser lightfrom the laser module is focused onto the specimen and illuminated, it entersthe excitation filter and hits the dichroic mirror.

Then the laser passesthrough the objective and hits in different focal plane. The emission beamagain passes through the objective and dichroic mirror and finally there is aemission filter which will prevent any other further

scattering there, thelaser light pass through it and reaches pinhole, which is a unique featureabout the confocal microscopy. Selected emission light will break through thepinhole aperture and hits the detector.

We can precisely focus particularregion of the cell which we are interested. Advantagesof Confocal microscopy:· Possible to get 3D image and 3Dreconstruction· Optical sectioning can be done withoutphysical contact· High resolution image (0. 1-0.

2? m)· We can choose the suitable excitationwavelength of the laser source. Limitationsof Confocal microscopy:· If it is a thick sample, tissue depthproblem occurs. When the laser hits the sample, intensity of the light/penetration power depends on the length/depth of the tissue. If it is thicker, the light will not able to penetrate the tissue.

Lot of energy loss occurs.· Choice of fluorophores is important· It is largely time consuming· Diffraction limits the image resolution.