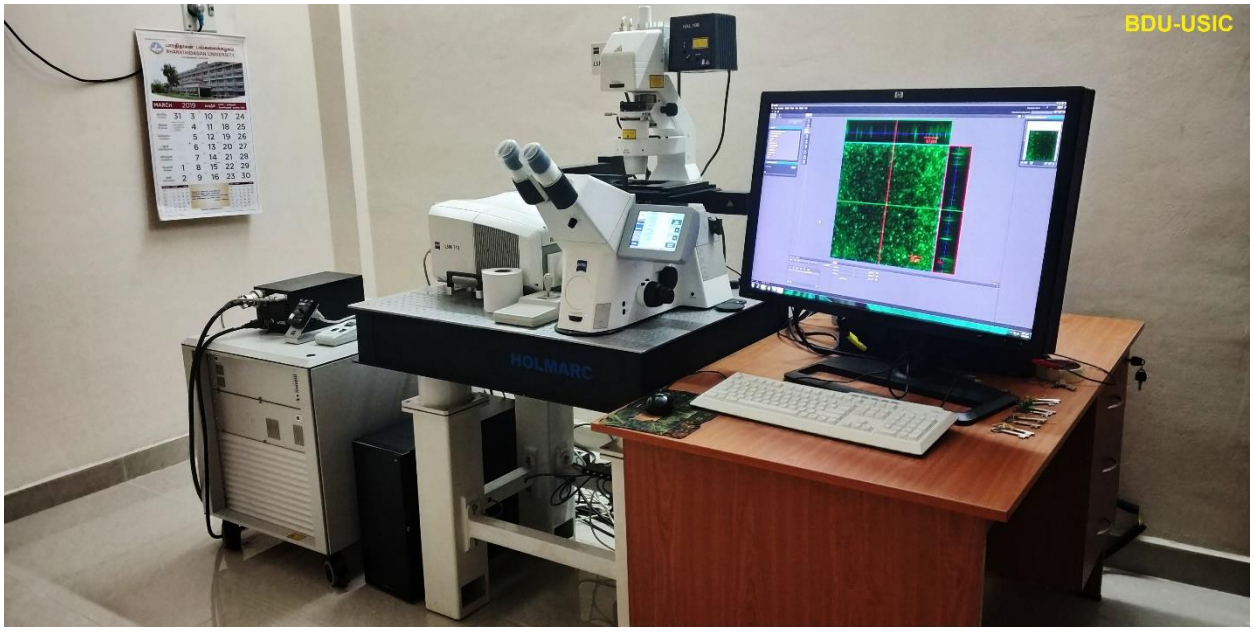
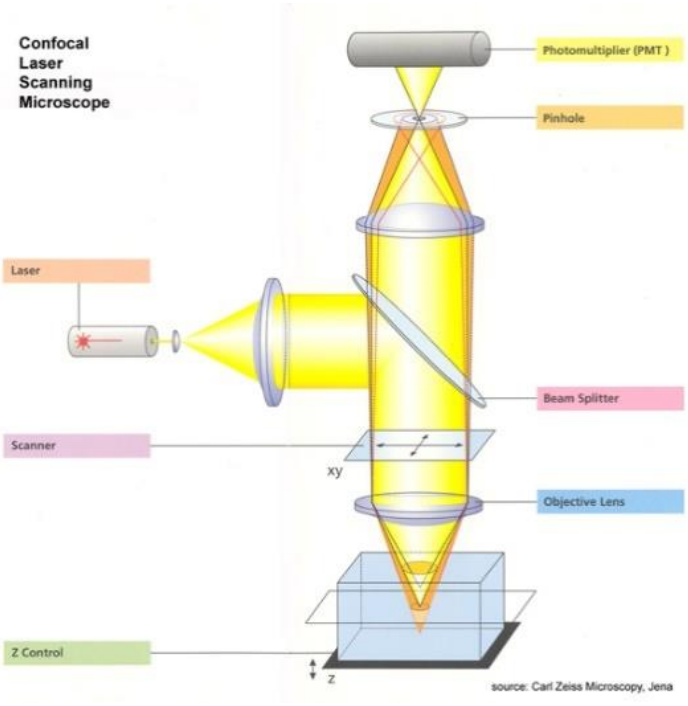
Confocal Laser Scanning Microscope

Confocal Laser Scanning Microscopy (usually shortened to just Confocal Microscopy) offers several advantages over conventional optical microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane, and the capability to collect serial optical sections from thick specimens. The basic key to the



confocal approach is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus. There has been a tremendous explosion in the popularity of confocal microscopy in recent years, due in part to the relative ease with which extremely high-quality images can be obtained from specimens prepared for conventional fluorescence microscopy, and the growing number of applications in cell biology that rely on imaging both fixed and living cells and tissues. In fact, confocal technology is proving to be one of the most important advances ever achieved in optical microscopy.



Confocal Microscopy comprises Fluorescent imaging applications of fixed and live cells, Long and Short-term Time-lapse imaging, Co-localization, Z-stacking & 3D reconstruction, FRET (sensitization and Photobleaching), FRAP, photoactivation, photoconversion, FCS/FCCS experiments.

Working Principle

- Similar to the widefield microscope, the confocal microscope uses fluorescence optics. Instead of illuminating the whole sample at once, laser light is focused onto a defined spot at a specific depth within the sample.
- This leads to the emission of fluorescent light at exactly this point.
- A pinhole inside the optical pathway cuts off signals that are out of focus, thus allowing only the fluorescence signals from the illuminated spot to enter the light detector.
- By scanning the specimen in a raster pattern, images of one single optical plane are created.
- 3D objects can be visualized by scanning several optical planes and stacking them using a suitable microscopy deconvolution software (z-stack).
- It is also possible to analyse multicolour immunofluorescence staining using state-of-the-art confocal microscopes that include several lasers and emission/excitation filters.

Features

- ✓ Bright field, Fluorescence and DIC observations and imaging
- ✓ Six-position motorized DIC nose piece, 6-position motorized FL filter wheel, with filter blocks for the imaging of samples with excitation range from 458nm to 633 nm.
- ✓ Following high resolution confocal microscopy grade Apochromatic Objectives all suitable for confocal fluorescence imaging, with DIC capability.
- ✓ 10X long working distance with capability of fluorescence work through plastic bottom culture plates. DIC/PlasDIC/Modulation contrast capability.

i. ~20/25X DIC Long Distance oil/water/glycerin for live cell imaging.
ii. ~40X oil DIC at least 1.2 NA.
iii. ~40X/63X water DIC at least 1.2 NA.
iv. 60/63X oil DIC at least1.4NA
v. 100X oil DIC at least 1.4NA

- ✓ High-speed XY scanner with total scan flexibilities of line, XY, XYZ, XYZT and XYZT? Combinations.
- ✓ Air-cooled multi-line Ar laser with 458/488/514nm and He-Ne laser with 543/633nm.
- ✓ Appropriate control PC with high-resolution graphics card and ~30" LCD TFT monitor with high-resolution.

Applications

- ➢ Confocal microscopy is broadly used to resolve the detailed structure of various **specific objects within the cell** through **Co-localization**.
- ➢ Similar to wide-field fluorescence microscopy, various components of living and fixed cells or tissue sections can be **specifically labelled** using **immunofluorescence** and then visualized in high resolution.
- ➢ Confocal microscopy enables the creation of sharp images of the exact plane of focus, without disturbing fluorescent light from the background or other regions of the specimen.
- ➢ Structures within thicker objects can be conveniently visualized using confocal microscopy. Microbes present inside the microtubules of the thick dental section are also visualized for **live / dead cell imaging**.
- ➢ **Biofilm formation** of several microorganisms can be studied with **Z** stacking. Furthermore, by stacking several images from different optical planes, **3D** structures can be analysed.
- ➢ **Time-lapse imaging** helps to capture the real-time events in cell apoptosis-related experiments in cancer studies and many more.

Details of Confocal Microscope

Brand	Carl Zeiss Microscopy GmbH, Germany
Model	LSM 710- Laser Scanning Confocal Microscope Workstation
Sponsored Agency	DST- PURSE & DST-SUPREME