Confocal microscopy for understanding fluorescently labelled biological samples

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Optical microscopy has been extensively used for studying samples from various fields, ranging from geology to life sciences [1]. In particular, advances in optics, electronics, light sources and digital imaging have increased the scope of microscopy remarkably for observing samples in actual conditions. In case of biological samples, various cellular and tissue entities can be labelled with fluorescent molecules, and this label can be used to monitor any changes that they might be undergoing. From an application standpoint, it is now possible to achieve diffraction-limited images with visible light [1]. In recent times, researchers have developed imaging techniques that can beat the diffraction limit of light using unique illumination and collection strategies [2].In particular, the improvement in the z-axis resolution using the confocal geometry opens avenues for optical sectioning of a sample with sub-200 nm resolution, wherein we can obtain the 3D image of the object. Confocal microscopy is a fast, laser-scanning fluorescence imaging technique that provides high-resolution and high-contrast images by eliminating out-of-focus light.

About the system in SATHI-CISCoM

At SATHI CISCoM at IIT Hyderabad, we have the Nikon Ti2-E confocal microscope equipped with NSPARC (Nikon Spatial Array Confocal) for super-resolution imaging and a TIRF (Total Internal Reflection Fluorescence) module for studying samples labelled with fluorescent dyes. This is achieved using point illumination and a pinhole aperture that allows only in-focus light to reach the detector. The Ti2-E confocal microscope features a large field of view (FOV) of 25 mm. The objective lenses used in this confocal microscope are 4x, 10x, 20x, 40x, 60x(oil) and 100x(oil). Chromatic aberration is reduced in confocal microscopy by using a nanocrystal coating on the objective lens. Equipped with the A1R HD laser-scanning confocal unit, the system offers six excitation lines (405, 445, 488, 514, 561, 640 nm), enabling a broad array of common fluorophores (DAPI, CFP, FITC, YFP, TRITC, Cy5). A hybrid scanning mechanism—combining high-speed resonant scanning (up to 30 fps at 512×512) with Galvano scanning (10 fps at 512×512; 4096×4096 spatial resolution)—provides flexibility in imaging speed and resolution. The detector assembly uses two traditional PMTs and two GaAsP detectors. The Perfect Focus System (PFS) is integrated into Nikon confocal microscopy systems. This PFS (Perfect Focus System) is an autofocus mechanism that automatically compensates for focus drift caused by temperature fluctuations or mechanical instability. It is especially useful during long-term time-lapse imaging in confocal microscopy (uses infrared tracking to maintain Z position). Ti2 E supports live-cell imaging with controlled temperature and CO_2, for prolonged experiments. It also supports various imaging modes, including large-area imaging, Z-stacking, time-lapse imaging, multipoint acquisition, multichannel imaging, and live-cell studies. Advanced techniques such as FRAP (Fluorescence Recovery After Photobleaching) and FRET (Förster Resonance Energy Transfer) can also utilized for functional imaging and molecular interaction studies.

Imaging fluorescently labelled samples using the confocal system

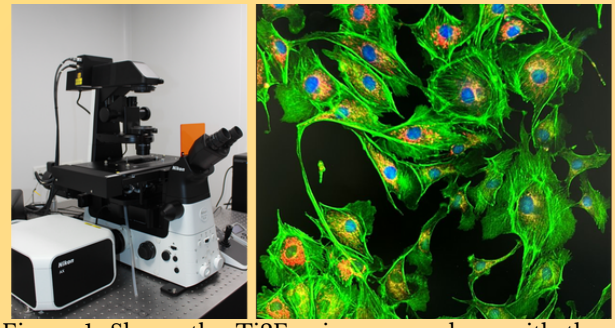

Figure 1. Shows the Ti2E microscope along with the image of a human cell line stained with three different dyes. Each of the dyes labels the nucleus, the mitochondria and the cytoskeleton of the cells. From this, we can easily observe the morphology of the different organelles and calculate the area of each of them.

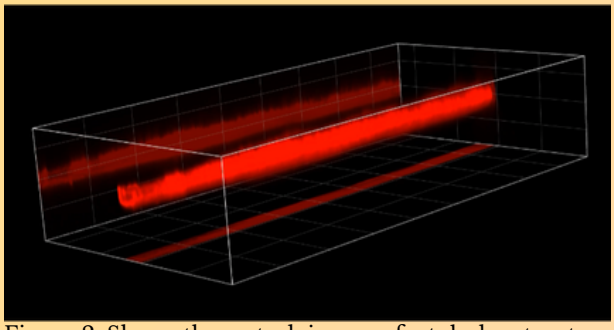

Figure 2. Shows the z-stack image of a tubular structure stained with Nile Red dye, wherein the dye only attaches to the surface of the structure. Using this it is possible to map the 3D surface topography of the sample.

References:

Characterization of Materials

1. Imagining the future of optical microscopy: everything, everywhere, all at once, Commun Biol., 6, p.1096 (2023).
2. Super-resolution microscopy demystified, Nature Cell Biology, 21, p. 72–84 (2019)

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