For M SC Biotech 2 sem By Dr Santosh Thakur

Confocal Microscopy

Introduction

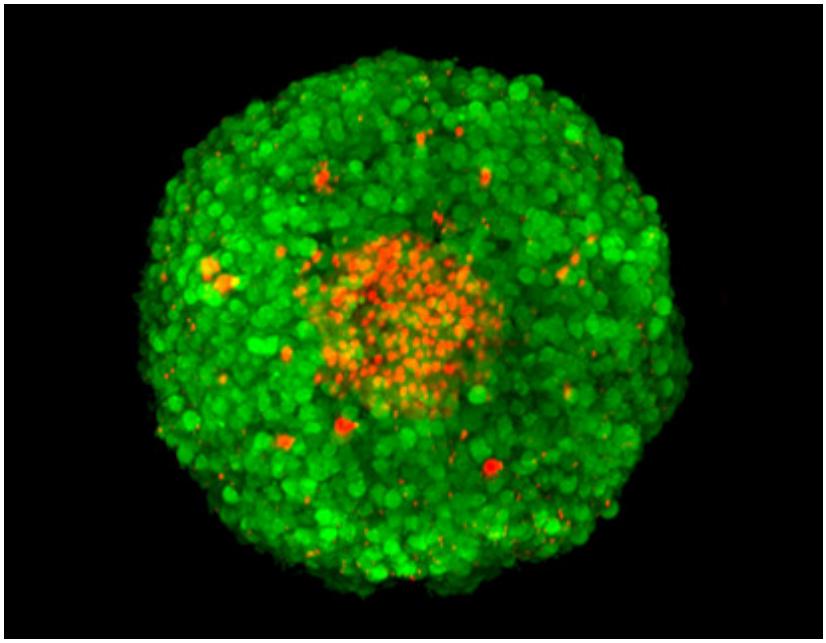
Confocal microscopy offers several advantages over conventional optical microscopy, including shallow depth of field, elimination of out-of-focus glare, and the ability to collect serial optical sections from thick specimens. In the biomedical sciences, a major application of confocal microscopy involves imaging either fixed or living cells and tissues that have usually been labeled with one or more fluorescent probes.

When fluorescent specimens are imaged using a conventional widefield optical microscope, secondary fluorescence emitted by the specimen that appears away from the region of interest often interferes with the resolution of those features that are in focus. This situation is especially problematic for specimens having a thickness greater than about 2 micrometers. The confocal imaging approach provides a marginal improvement in both axial and lateral resolution, but it is the ability of the instrument to exclude from the image the "out-of focus" flare that occurs in thick fluorescently labeled specimens, which has caused the recent explosion in popularity of the technique. Most current confocal microscopes are relatively easy to operate and have become part of the basic instrumentation of many multi-user imaging facilities. Because the resolution possible in the laser scanning confocal microscope (**LSCM**) is somewhat better than in the conventional widefield optical microscope, but still considerably less than that of the transmission electron microscope, it has in some ways bridged the gap between the two more commonly used techniques

Applications:

Confocal microscopy is broadly used to resolve the detailed structure of specific objects within the cell. Similar to widefield fluorescence microscopy, various components of living and fixed cells or tissue sections can be specifically labeled using immunofluorescence, for example, and then visualized in high resolution.

As a distinctive feature, confocal microscopy enables the creation of sharp images of the exact plane of focus, without any disturbing fluorescent light from the background or other regions of the specimen. Therefore, structures within thicker objects can be conveniently visualized using confocal microscopy. Furthermore, by stacking several images from different optical planes, 3D structures can be analyzed. The sample penetration depth is limited, however, when using confocal microscopy. Thicker objects, like large spheroids, organoids, tissue, and small animals, should instead be optimally imaged using two-photon microscopy or LSFM.



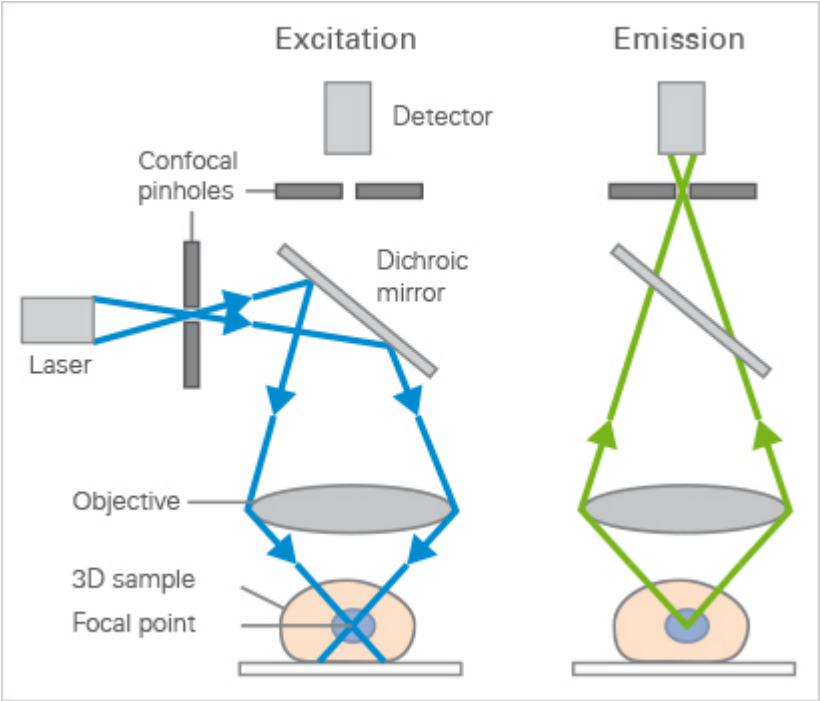
Z-stack of an FDA/PI-stained MCF-7 spheroid, acquisition by confocal microscopy. Green: FDA-stained living cells. Red: PI-stained dead cells in the necrotic center of the spheroid.

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 analyze multicolor immunofluorescence stainings using state-of-the-art confocal microscopes that include several lasers and emission/excitation filters.



Excitation and emission light pathways in a basic confocal microscope configuration.