Welcome to the Confocal Laser Scanning Microscope Core Facility. The facility enables local researchers to incorporate high resolution confocal techniques in their research programs and provides instruction for use of the LSM.

Location:
Verfügungsgebäude für Forschung & Entwicklung

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Equipment: The equipment includes a Zeiss Confocal LSM510-UV equipped with the following lasers and their lines:

Argon-UV (351nm, 364 nm) -Hoescht, -DAPI
Argon Laser (458nm, 488nm) -CYP, -GFP, -FITC, -Alexa 488
HeliumNeon (543nm) -TexasRed, -Cy3, -Alexa546
HeliumNeon (633nm) -Cy5, -Alexa633, -Toto3

The system includes a fully integrated workstation that incorporates image acquisition and image analysis software with high resolution confocal optics:

Three fluorescence detectors.
Nomarski optics allow superimposition of signals from three fluorophores on a DIC image.
Zeiss software for reconstruction of 3-D images, presentation of image "galleries", Z-sectioning, time series, etc.
The Zeiss Axiovert Inverted Scope used by the system has 10x, 20x 40x and 63Xoil, 63xwater objectives and a Z-axis motor. All functions are computer controlled.
A temperature controlled tissue culture stage, which holds chambers with cover slip bottoms and applies CO_2.

Key sevices provided by the LSM facility

- Four channel fluorescent imaging plus differential interference contrast optics in live and fixed specimens
- Three dimensional acquisition and conversion to 360° projections
- Long and short term live imaging using time lapse acquisition
- FRAP (Fluorescence Recovery After Photobleaching) analysis
- FRET (Fluorescence Resonance Energy Transfer) analysis
- Reagents for imaging specific organelles, subcellular compartments, and physiological processes

A Few Advantages of CLSM

1. Clearer images

Due to its point illumination/detection properties, out of focus light is excluded from reaching the detector. Because of this, only in-focus light is detected, hence in focus images are collected.

2. Increased sensitivity

In confocal microscopic systems fluorescence from the sample is detected using highly sensitive photomultiplier tubes (PMTs). This is advantageous in that samples of low intensity can be imaged. Furthermore, for samples that are sensitive to photobleaching, lower excitation intensity can be used hence allowing for longer imaging times.

3. Reduced photobleaching

Because of the use of point illumination light sources (lasers) to excite the sample, only small areas of the samples are photobleached.

4. Three-dimensional reconstruction

Due to its ability to perform optical sectioning, thicker samples can be imaged and reconstructed in an in-focus three-dimensional manner using image analysis software. These images can be displayed as rotating movies as well as stereo-images.

5. More accurate quantitation

The low incidence of out of focus light in the digital images generated by CLSM enables more accurate quantitation of images. Quantitating relative fluorescence levels within an image can be achieved using commercial or freeware image analysis software.

6. Multiple simultaneous analyses

Multiple fluorescence detectors allow simultaneous analysis of multiple cellular properties and/or markers. The range of useful fluorochromes is further expanded in instruments utilizing multiple lasers. For further information on choosing compatible fluorochrome sets, see "Fluorescence in Cytometry".