

## **Confocal Laser Scanning Microscope**

## **Characterization Laboratory**

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#### Lab. 07

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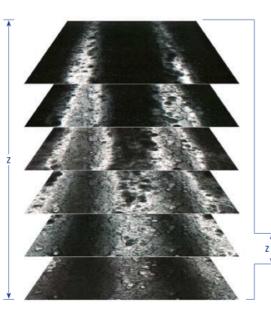
#### Applications

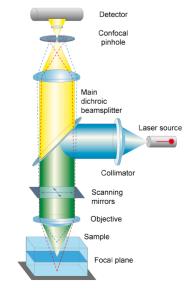
Morphological, topographic and structural characterization of microstructured samples from different fields: material science, microelectronics, geology, biology, chemistry.

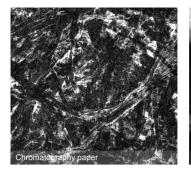
Co-localization analysis (detection of emissions from two or more fluorescent molecules)

#### **Confocal Principle**

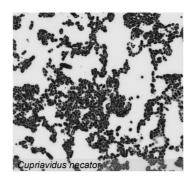
The LSM 700 is a light microscopy system that uses laser light in a confocal beam path to capture defined optical sections of the material sample and combine them into a three-dimensional image stack. The basic principle behind confocal microscopy is the use of spacial filtering to generate a focused point of illumination combined with a pinhole at the image plane in such way that the outof-focus light does not reach the detector. Only light focused at the pinhole passes through it, all other light is scattered. Since this solution only provides information about a single point at one time, in order to build an image the focused spot of light must be scanned across the specimen. The precise optical sectioning of thick specimens is provided by a motorized z-axis drive. It is thereby possible to generate precise threedimensional data sets that can be reconstructed into models of the sample in 3D space. This provides structural properties and reveals detailed information regarding the structures localization within the sample.













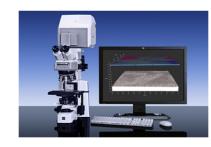
# Technical specifications

LSM 700 Laser Scanning Microscope from Carl Zeiss

- Microscope Axio Imager.Z2m (Upright stand)
- Z drive step motor (smallest increment of 10nm)
- Motorized XY scanning stage
- Motorized master pinhole
  (diameter continuously adjustable)
- Reflected-light objectives lenses of magnification 10X, 20X, 50X, 100X and 63X for use with immersion oil.
- Pigtail-coupled solid-state laser with polarization-preserving single-mode fiber. Customizable intensity adjustment of the included laser lines: 405 nm (5 mW), 488 nm (10 mW), and 555 nm (10 mW).
- Two confocal detection channels (reflection/fluorescence), each with high-sensitivity PMT detector (spectral increment: 1 nm)
- Variable short pass beam splitter for precise tuning of wavelength at which signals are split (splitting possible between 420 and 630 nm, minimum step: 1 nm)

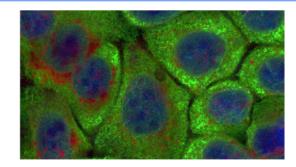
### **Additional features**

- Axio Imager.Z2m upright stand for reflected light, bright and dark field, with highresolution AxioCam microscope camera for acquisition of optical images.
- Confocal capture modes include: Spot, Line/Spline, Frame, Z stack, and Time-Lapse series.
- Lambda stack acquisition: highly light-efficient detection strategies and spectral imaging.
- Image presentation modes include: orthogonal view (XY, XZ, YZ in a single presentation), Cut view (3D section made under a freely definable spatial angle), Depth coding (pseudo-color presentation of height information), Topographic view (3D reconstruction of the object's topography), and 3D view.
- Geometric parameters (length, width, height, profile angle, area)
- Roughness parameters (mean height, mean deviation, peak height, valley depth)
- Correlative Microscopy Shuttle and Find imaging of sample positions in the laser scanning microscope for reproducible repositioning after transfer to scanning electron microscope (SEM).





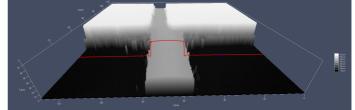
#### Fluorescence



Co-localization analysis of cancer cells (green) incubated with gold nanoparticles (red).

#### Topographic

3D surface topography with section of measured profile of a PDMS microfluidic channel.



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