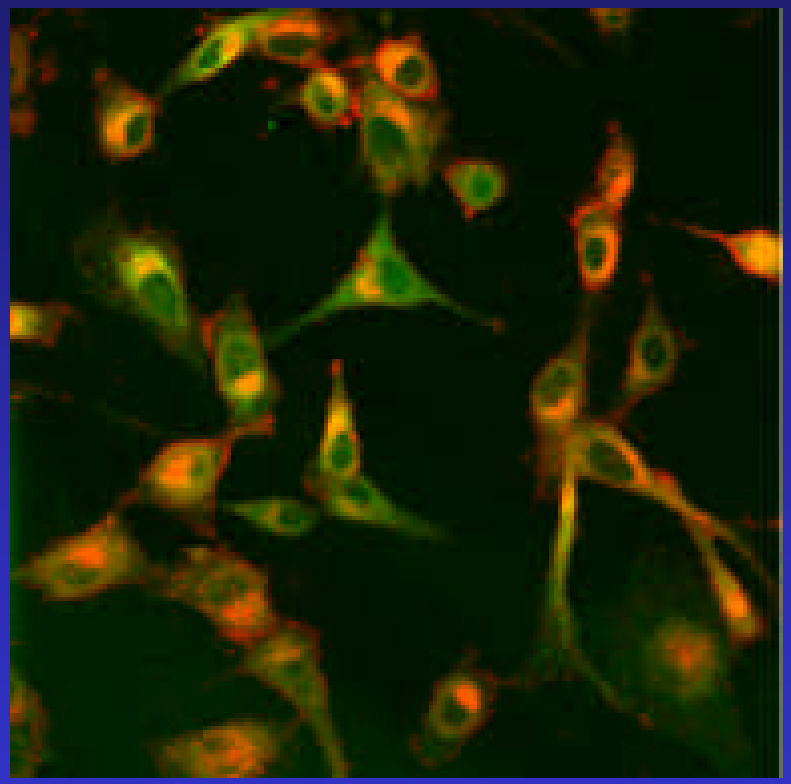
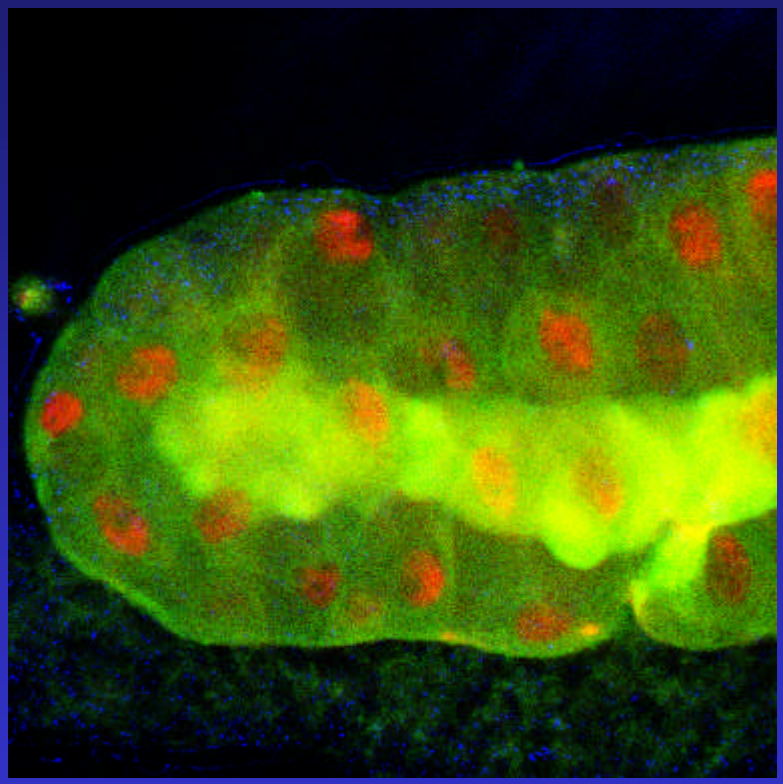
Laser scanning multispectral confocal microscopy

Areas of activity

multi-parametric characterisation of biological systems in physiological conditions, material analysis, fusion of molecular / single-cell / tissue techniques, emission fingerprinting, FRAP, FRET

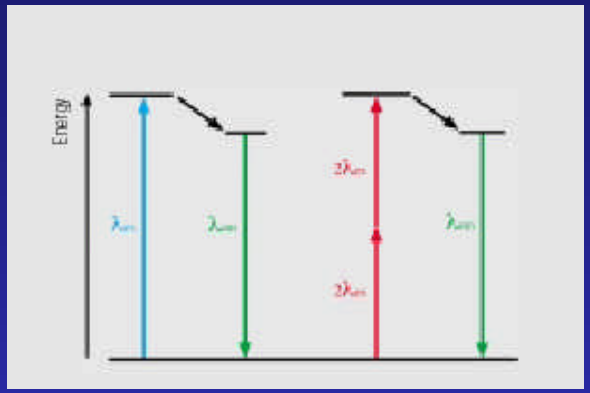
Laser confocal microscopy

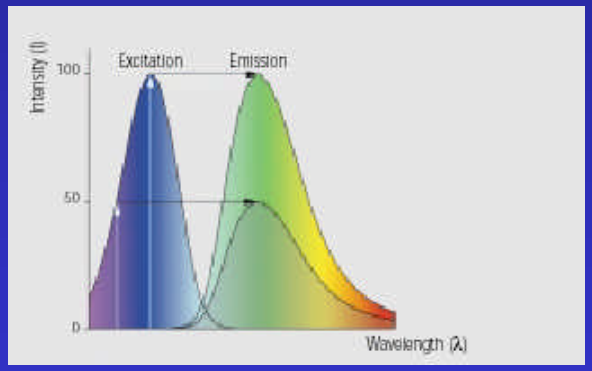


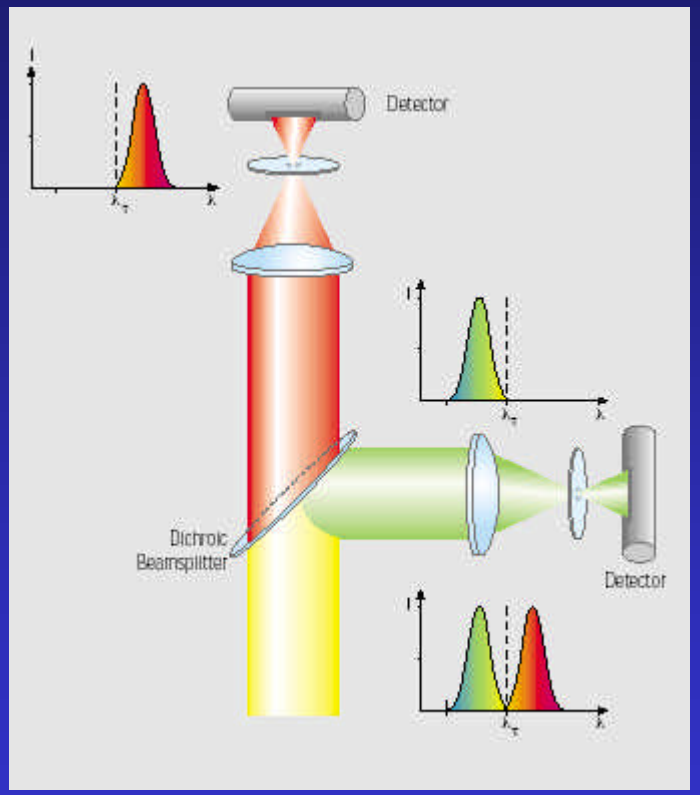


Fluorescence microscopy

Laser confocal microscopy

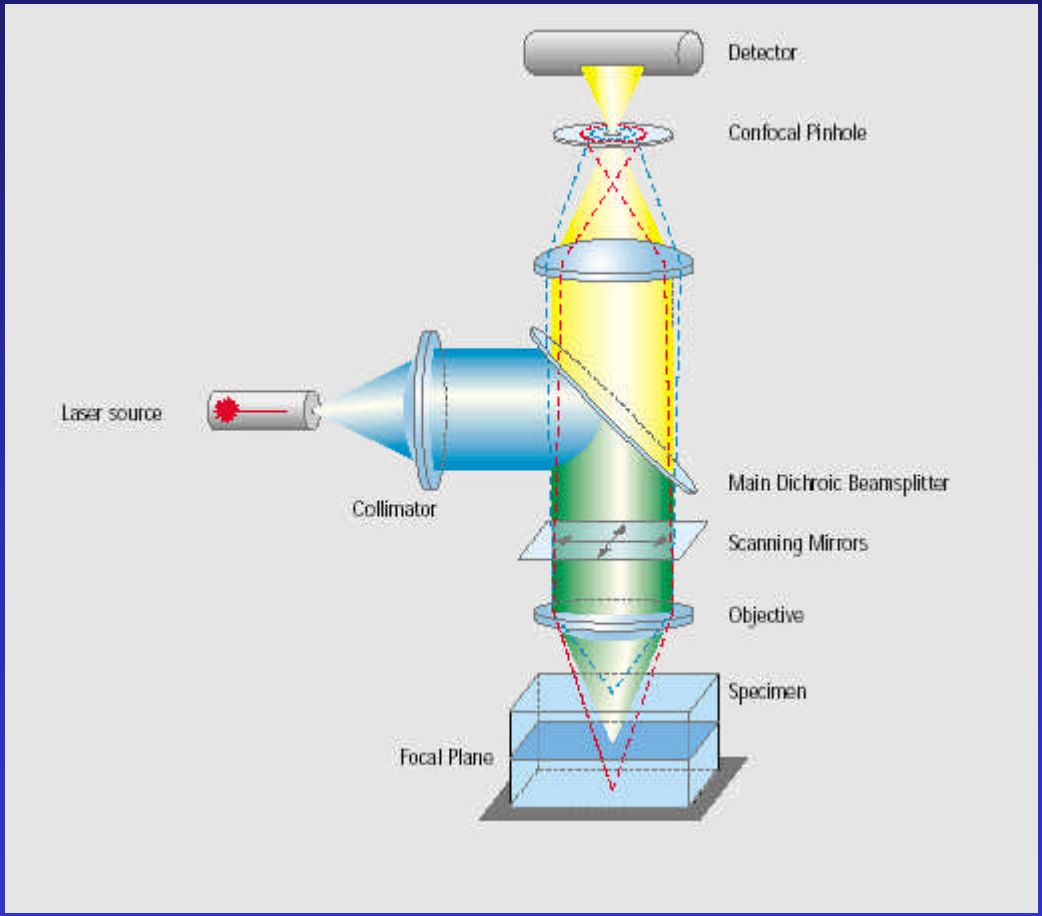






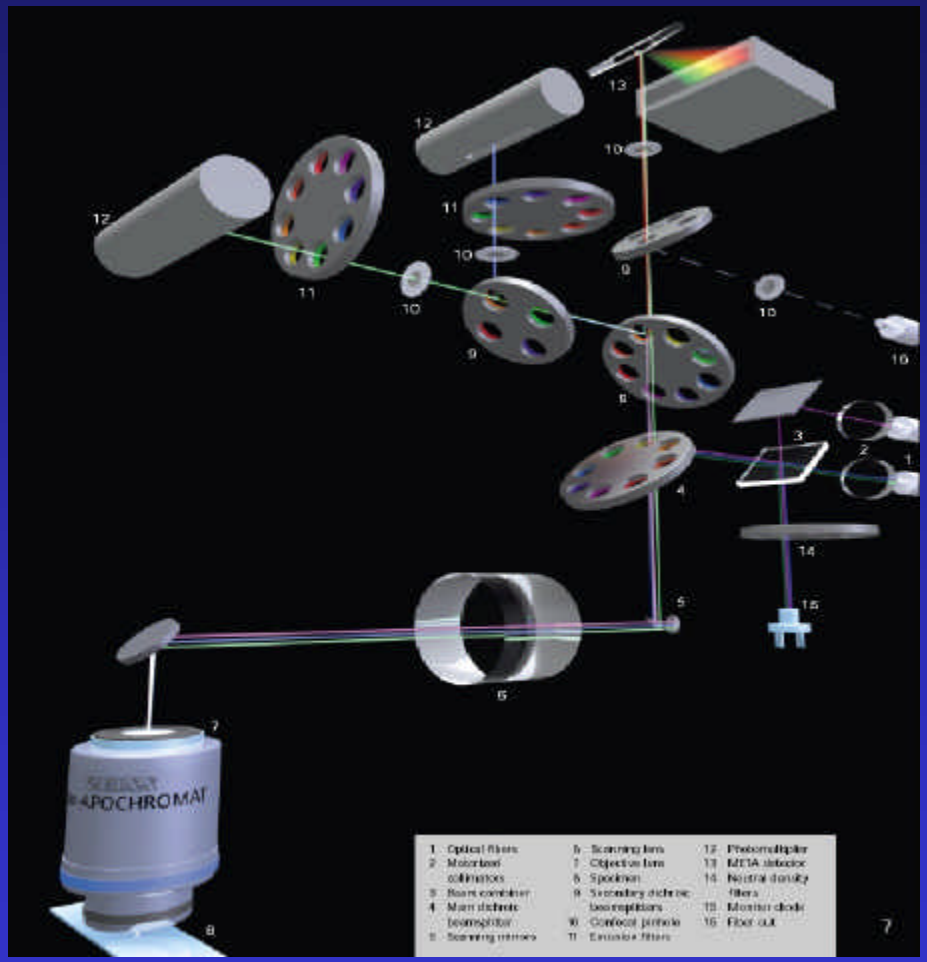
Fluorescence microscopy

Laser confocal microscopy



Basic principle of confocal microscopy

Laser confocal microscopy



Optical scheme of LSM META 510

Lasers:

Ar laser (458 nm, 477 nm, 488 nm, 514 nm) 30 mW

HeNe laser (543 nm) 1mW

HeNe laser (633 nm) 5 mW

Data Depth:

Selectable between 8 bit and 12 bit

Acquisition modes:

Spot, line/spline, Frame, Z Stack, Lambda Stack, Time Series and combinations –xy, xyz, xyt, xyzt, xz, xt, xzt, Spot-t, x-lambda, xy-lambda, xyz-lambda, xyt-lambda, xyzt-lambda, xz-lambda, xzt-lambda, averaging, summation, step scan – configurable

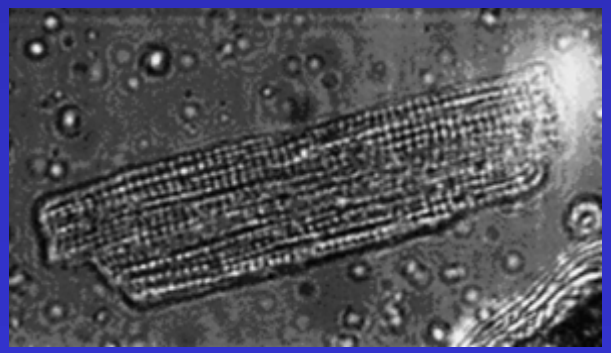
Mapping of physiological processes in living cells

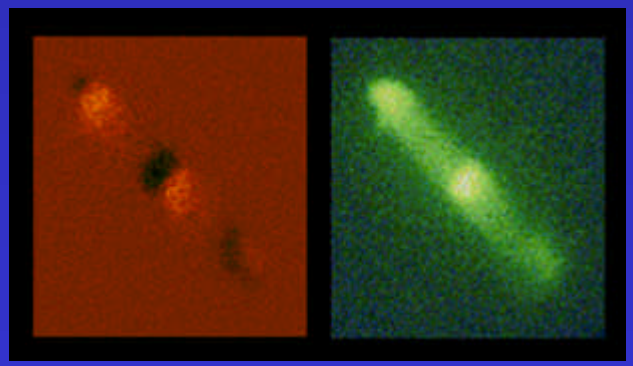
sample conditioning

temperature control
incubation / perfusion
under physiological conditions
+ Facility for cell cultivation

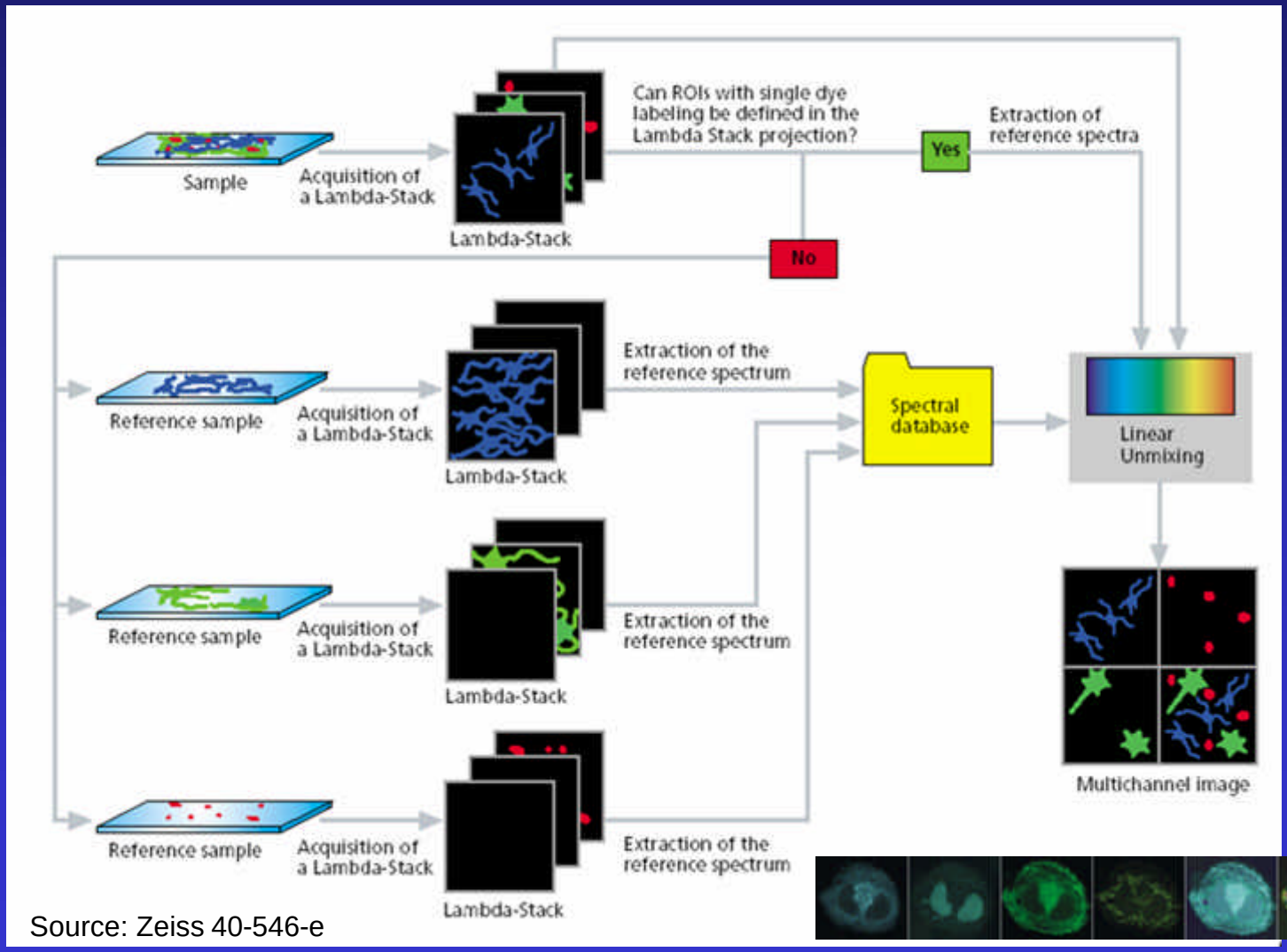
automatisation

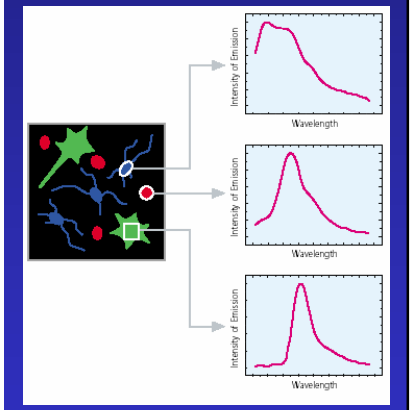
Programmable experiment protocols
and data-processing procedures





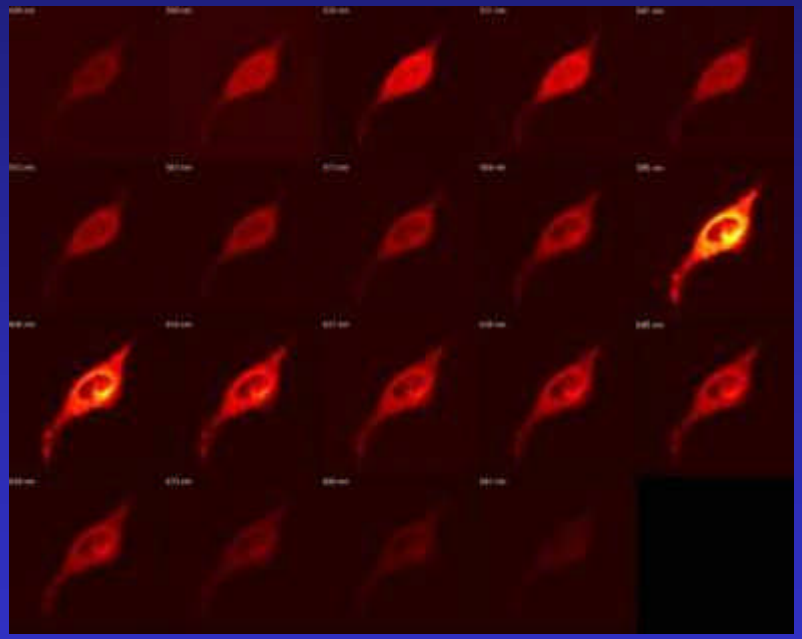
Emission fingerprinting (? microscopy)



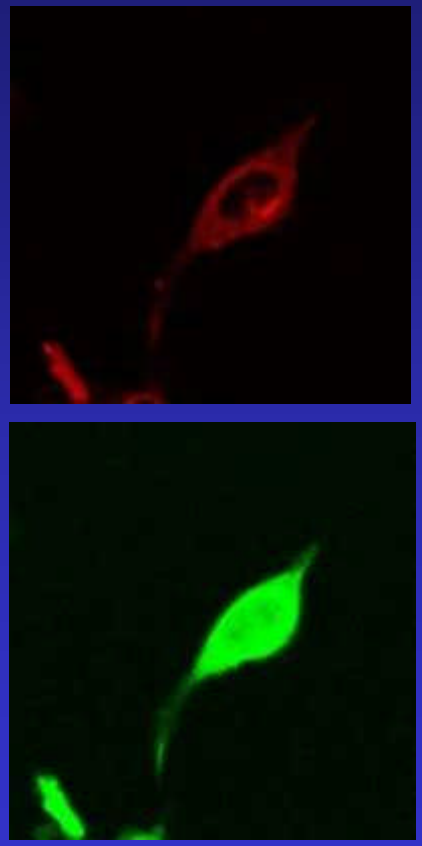


Source: Zeiss 40-546-e

Distribution of compounds in cells

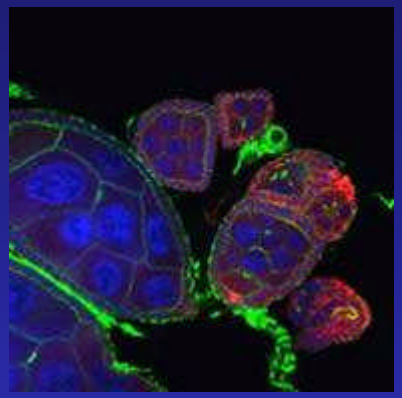


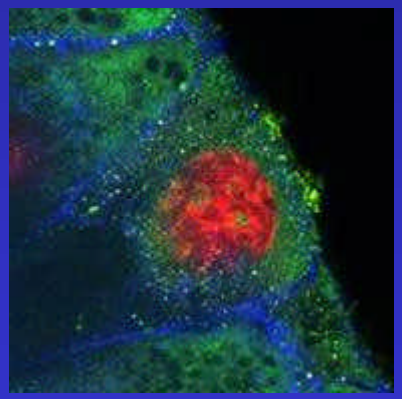
Spectrally resolved images



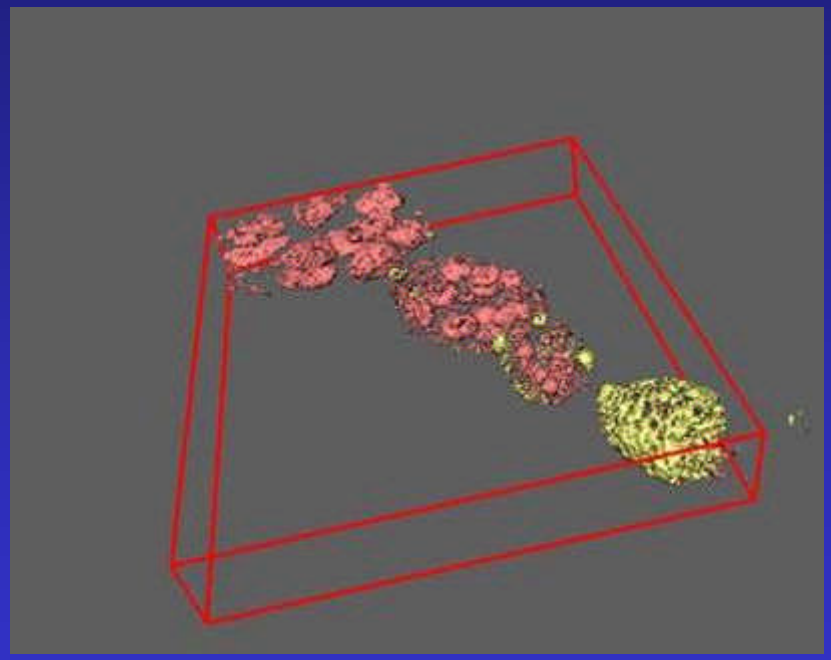
Unmixed images

Expression of various proteins and their location in cells



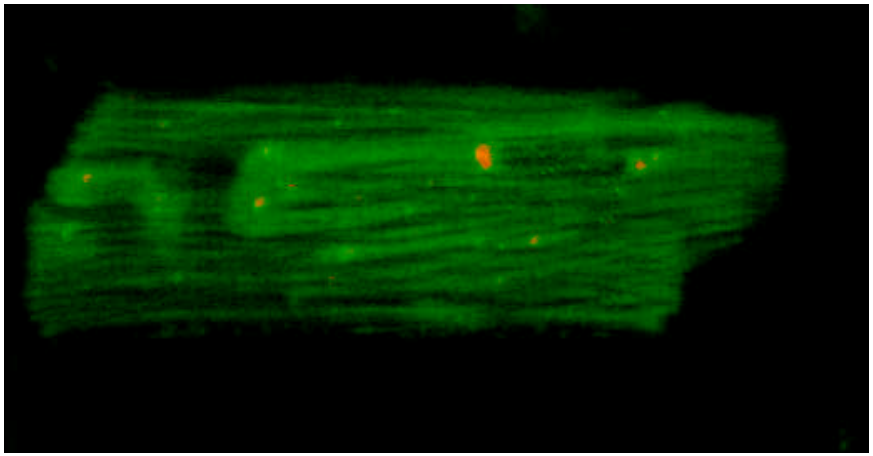


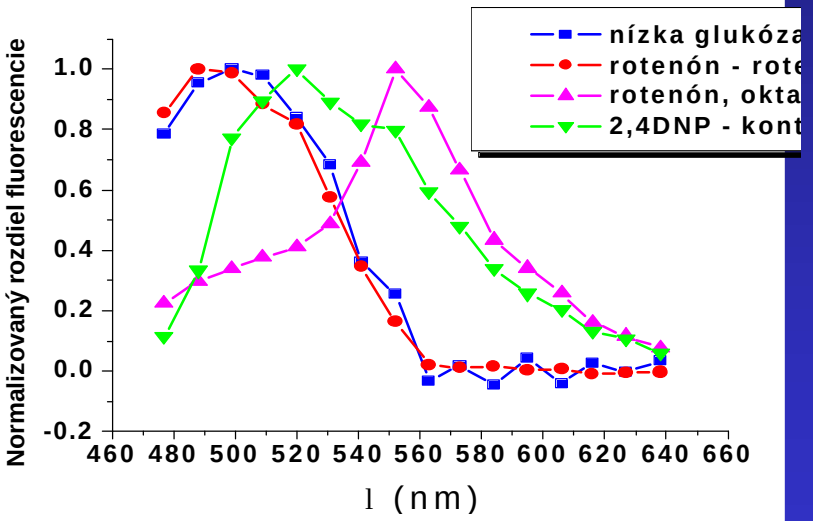
3D stack of images



3D reconstruction

Autofluorescence of cells





J. Kirchnerova, ILC

Visualization tasks

3D microscopy visualization (interactivity and transfer function design)

Feature tracking, segmentation

Visualization of multicolored (spectral) data sets

Measurements – volume, surface, length

Deconvolution in 3D space, difficult to measure PSF for biological samples due to variations in refractive indexes over the sample and light scattering of ligth

Colocalization in 3D space or 3D+time space

Problems in visualization of confocal datasets

Asymmetrical shape of PSF followed by distortions of 3D shapes

Fluorescence attenuation in depth due to photo bleaching

Variable contrast, contrast can degrades with increasing depth of penetration owning to light scattered and absorbed outside focal region.

Noise, fluorescently-labeled samples characteristically have low signal levels

Unknown structures

Large multidimensional datasets