EMBO practical course; FRET, FLIM, FCS, FRAP and 3D Imaging; Application to Cell and Developmental Biology

April 13th-24th 2009, Biopolis, Singapore.

Topics to include:

- Basic Microscopy
- Fluorescence Microscopy
- Confocal Microscopy
- SPIM
- Image Processing
- Deconvolution
- Imaging of Model Organisms
- FRET
- FRAP
- FLIM
- FCS and FCCS

Course Organizers:
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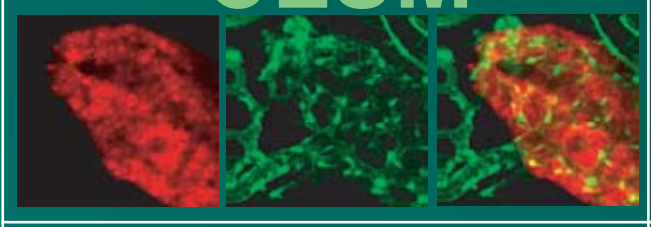
Malte Wachsmuth (EMBL, Germany)

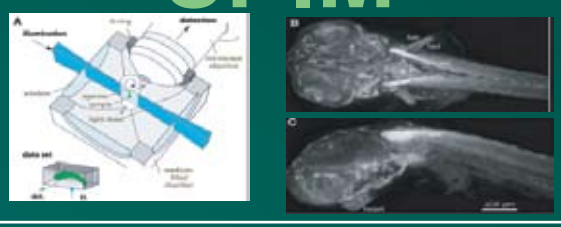
CLSM

CLSM (Confocal Laser Scanning Microscopy), CFM (Confocal Fluorescence Microscopy), or LSM (Laser Scanning Microscopy): In the CFM 3D images can be acquired by scanning a laser focus through a sample and recording fluorescence only from the focal region with the help of a pinhole. This detection pinhole discriminates against the fluorescent light emitted outside the focal plane. CLSM thus allows recording a 3D image limited only by the optical resolution. By using different lasers for excitation different fluorophores can be excited and differently stained regions in a sample can be visualized.



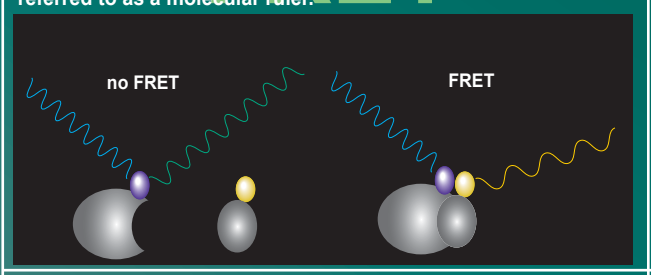
SPIM

Selective Plane Illumination Microscopy (SPIM) is a novel technique that allows imaging of living systems in 3D over long periods of time. Its principle is based on the illumination with a laser light sheet and the registration of the signal (fluorescence or scattering) by a CCD camera orthogonal to the illumination sheet. By translating and rotating the specimen 3D image stacks are obtained. Artifacts due to tissue scattering and absorption are considerably reduced. SPIM produces 3D images with a large penetration depth and isotropic resolution. Both points are vital for 3D live specimen imaging.



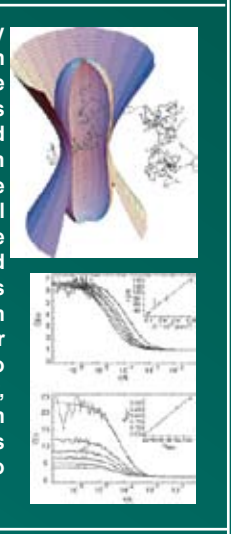
FRET

Fluorescence Resonance Energy Transfer (FRET) is the non-radiative energy transfer between an excited donor fluorophore and a fluorescent acceptor molecule. The transfer of energy leads to a decrease in the brightness of the donor molecule with a concomitant increase in the brightness of the acceptor molecule. Since FRET can only take place when molecules are closer than about 10 nm, FRET is often used to determine molecular interactions in vivo and in vitro. Due to the strong distance dependence of the FRET process it is often referred to as a molecular ruler.



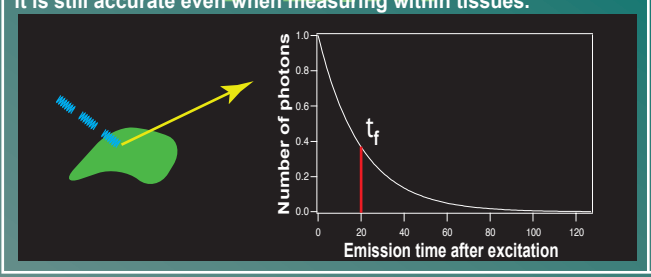
FCS

Fluorescence Correlation Spectroscopy (FCS) or Fluorescence Cross - Correlation Spectroscopy (FCCS) records the fluorescence intensity fluctuations caused by fluorescently labeled molecules transiting a small observation volume (usually a confocal volume). The fluctuations contain information about all processes causing the fluorescence fluctuations, i.e. characteristic time and frequency of occurrence and thus allows the determination of diffusion coefficients, concentrations and other related parameters. FCCS follows two different spectral bands independently, and thus allows the determination whether the signal of the two molecules are correlated and thus whether the two molecules interact.



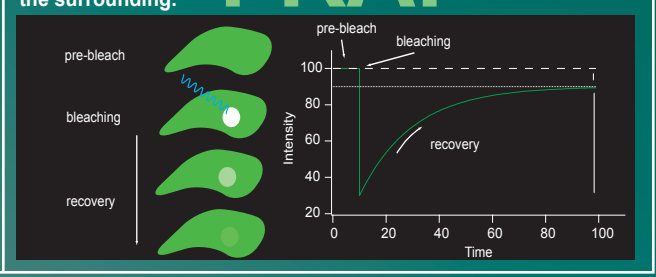
FLIM

Fluorescence Lifetime Imaging Microscopy (FLIM) measures the fluorescence lifetime of fluorophores at all imaged pixels in a sample. Since the fluorescence lifetime, i.e. the lifetime of the fluorophore in the excited state, depends critically on its locally surrounding medium, FLIM can determine FRET (which shortens the lifetime of the donor), pH, polarity and other parameters which influence the electronic state of the fluorophore. Since FLIM is independent of intensity and determines lifetimes, scattering and absorption do not influence its measurement and it is still accurate even when measuring within tissues.



FRAP

Fluorescence Recovery After Photobleaching (FRAP) is conducted by bleaching fluorophores in a defined area of interest with a strong, short laser pulse. The region is then observed during its recovery of fluorescence due to the exchange of bleached molecules for intact molecules of the surrounding. Two important parameters can be determined. Firstly, the rate of recovery which is related to the diffusion coefficient in the medium. Secondly, one can determine whether some molecules are immobile and thus do not exchange with the surrounding.



Instructors to include:
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For Registration and more information please visit http://cwp.embo.org/wpc09-01/

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