Imaging facilities at WUR

WAGENINGEN UNIVERSITY
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‘Advanced light microscopy facilities at Wageningen UR’

Lunch meeting organized by Cat-Agro Food

Programme Thursday 13 June 2013

12.00 Welcome and sandwich lunch

12.10 Introduction to light microscopy – *Norbert de Ruijter (Cell Biology and WLMC)*

12.30 Interactive presentations about recently purchased state-of-the-art light microscopy equipment and their applications:

12:30 BD Pathway bio-imager: high throughput high-content imaging and analysis of live and fixed cells –*Jurgen Karczewski (Host Microbe Interactomics)*

12:50 Spinning disk confocal microscopy: ideal for imaging of living cells or weak fluorescent signals –*Norbert de Ruijter (Cell Biology and WLMC)*

13:10 Multi-mode confocal laser scanning microscopy: with fluorescence lifetime imaging microscopy (FLIM) option for the detection of protein interactions in living cells – *Jan Willem Borst (Biochemistry and MSC)*

13:30 Single molecule total internal reflection fluorescence microscopy (smTIRF): Studying (dynamic) interactions in and between single proteins - *Johannes Hohlbein (Biophysics and MSC)*

14.00 End of the programme

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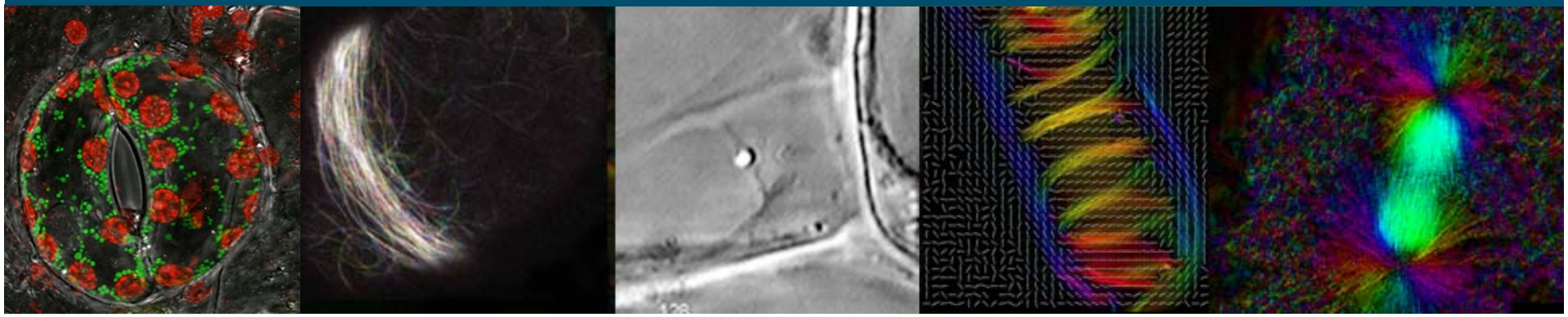
Introduction to light microscopy

Norbert de Ruijter
Laboratory of Cell Biology
WUR – PSG
www.clb.wur.nl



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WLMC
Wageningen Light Microscopy Center

Aims

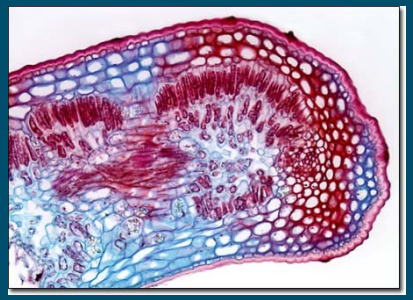
- Refresh your basic knowledge on microscopy/ fluorescence/ confocal

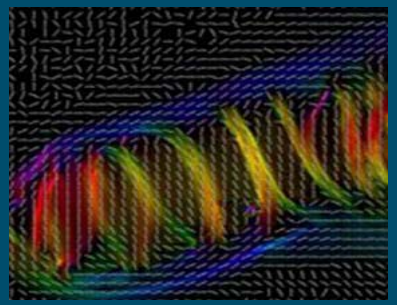
- What equipment best serves my research needs?
- Where can I get assistance / access to microscopes?
- What are the main Imaging Facilities within WUR?

- Explain possibilities with latest CAT AGRO Food investments
  in LM: 4 small presentations

LM techniques

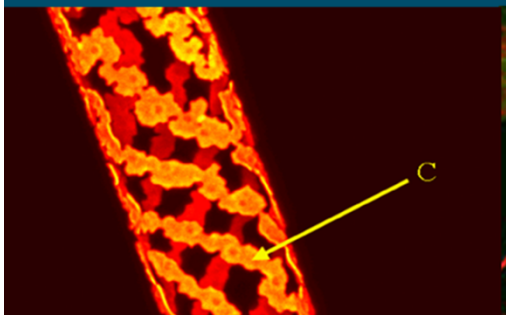
- Bright field Imaging
  - BF: Contrast by variations in light absorbance
  - DIC/Phase: Contrast by variations in interference/breaking index
  - Polarization microscopy: Contrast by birefringent polymers-crystals



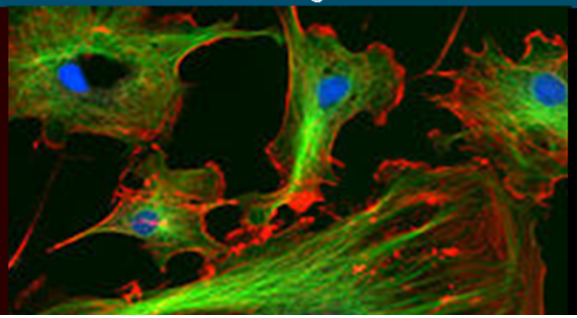


- Fluorescence Imaging
  - FL: dark field technique based on FL excitation / emission
  - Many ways for localization-dynamics-quantitative studies

Auto-FL chloroplasts in Spyrogyra



Immuno-FL labeling in fibroblasts



In situ hybridisation with FL probes

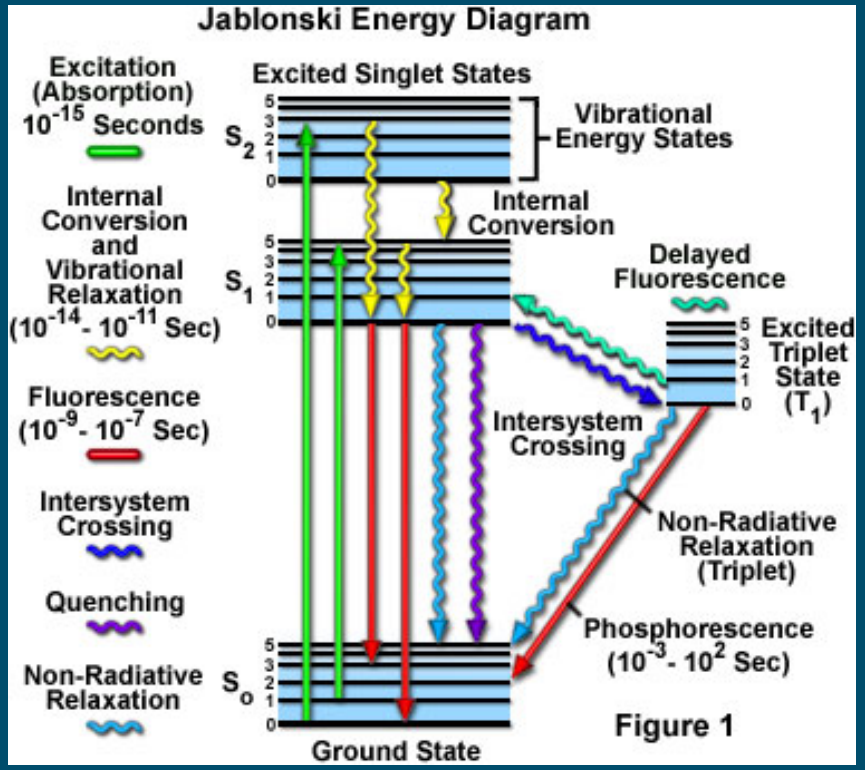
Fluorescence: Jablonski energy diagram

Discriminate 3 steps:

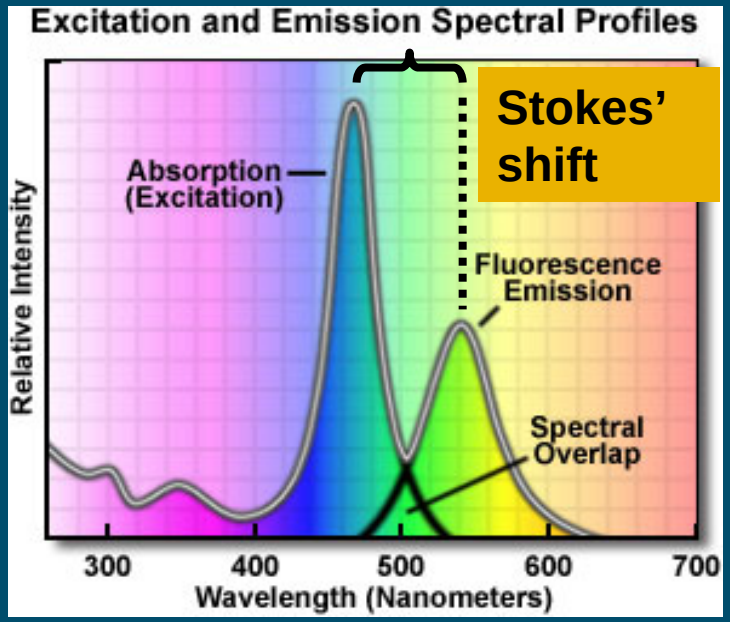
1. A fluorophore absorbs a high energetic photon (light particle)

2. An electron comes temporarily in an excited vibrational energy state (with short lifetime).

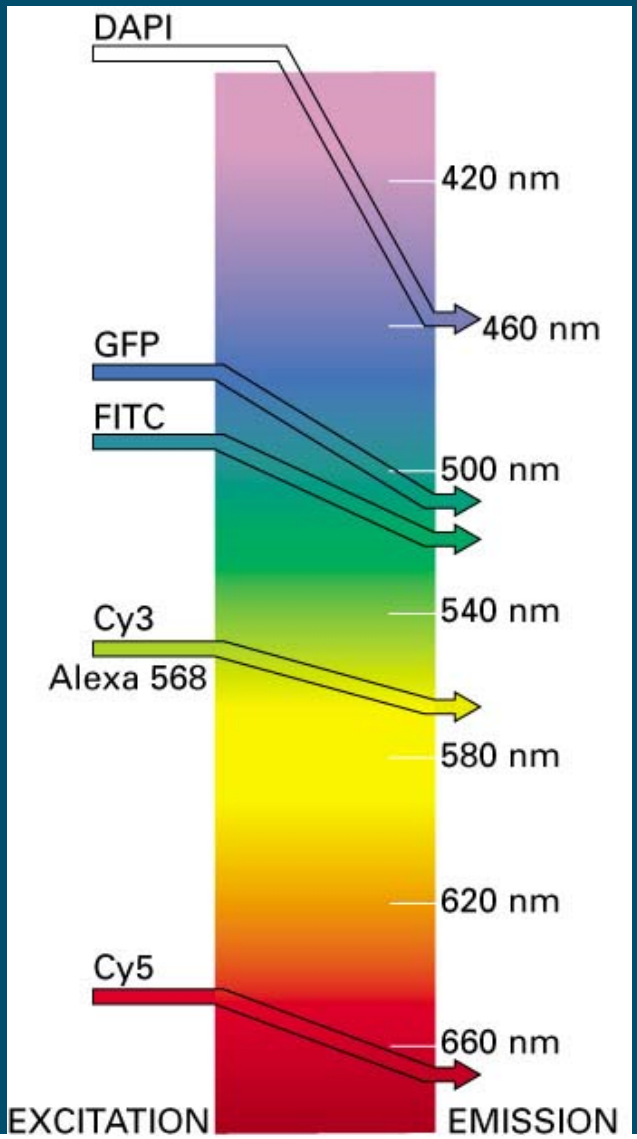
3. A photon of lower energy is emitted, returning the fluorophore back to ground state (S_0) = Fluorescence!



Fluorescence: photons are emitted with higher wavelength but lower energy

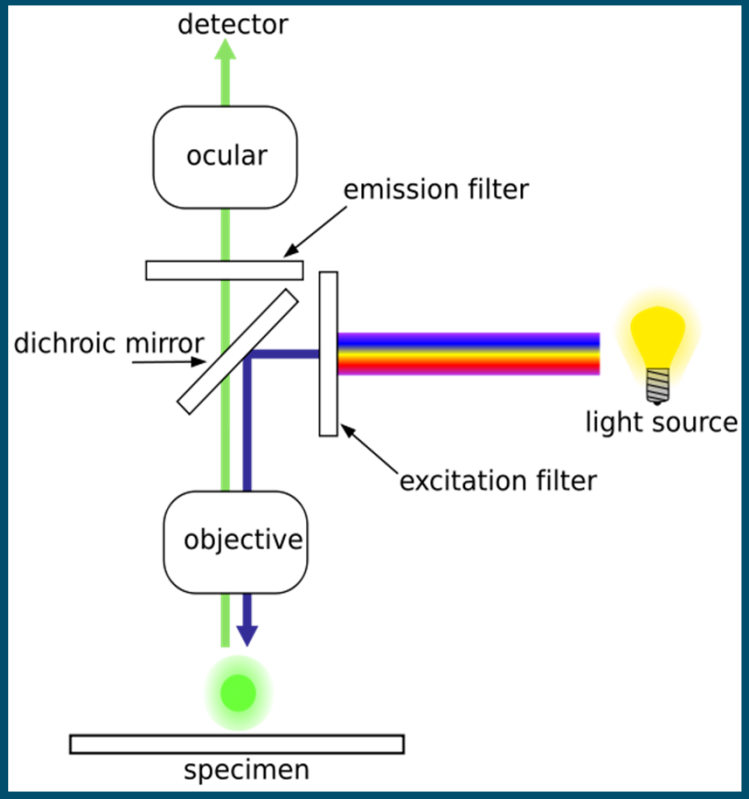


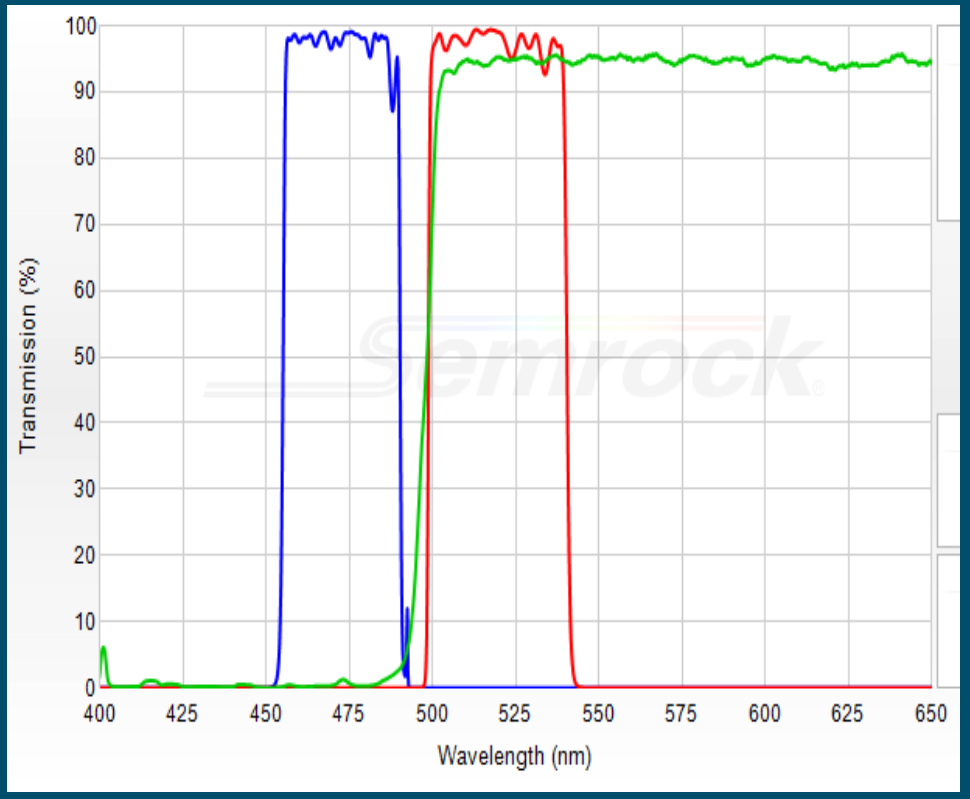
Higher energy



Lower energy

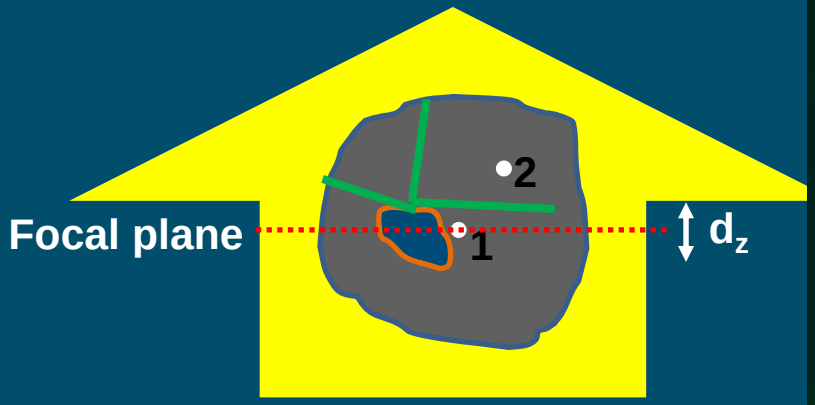
Filters and dichroic mirror allow to select specific emission

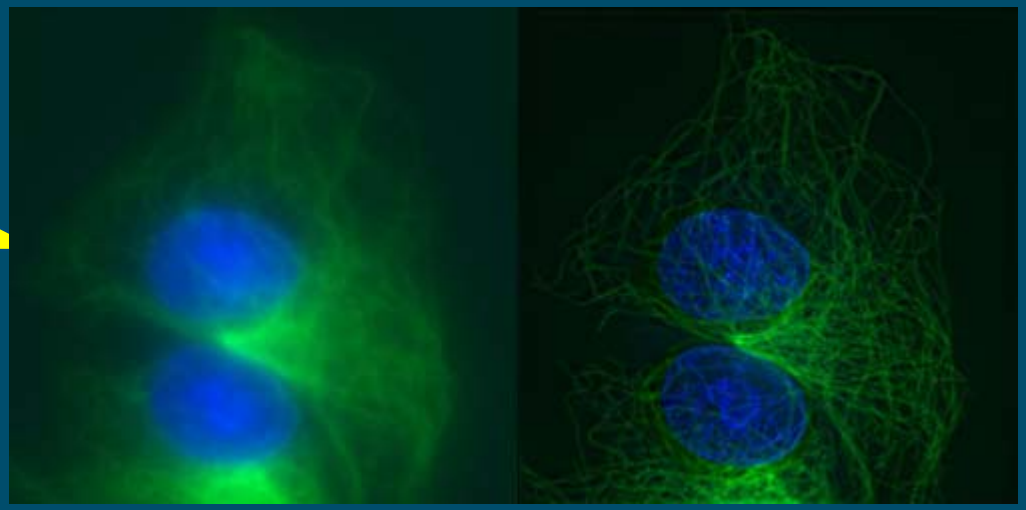




Excitation light does not reach the detector, since dichroic mirror reflects excitation and transmits emission light. You need much intensity in a specific wavelength band (LED/laser) for optimal excitation.

Out-of-focus blur





A cell with stained DNA (blue) and microtubules (green)

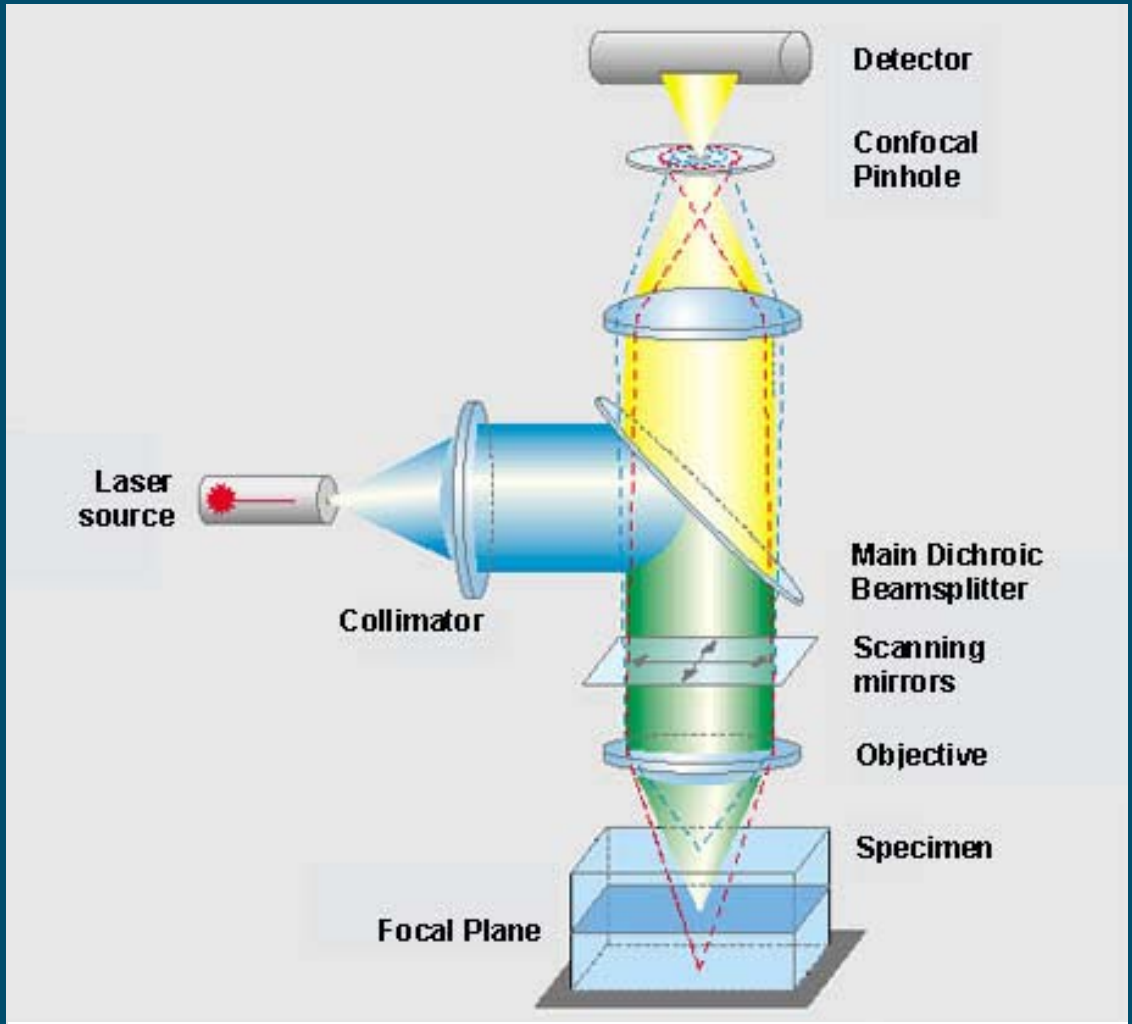
- Cell is illuminated with excitation light from below: Positions 1 and 2 at different heights in the sample receive equal excitation intensity and fluorophores become excited.
- Emission from the focal plane is detected sharp (in focus) on (PMT/CCD) detector. However, emission light from out-of-focus planes superimposes a blur that prevents us from seeing detail in the sample.

Remove out-of-focus blur by optical sectioning

- Confocal Laser Scanning Microscopy (JW Borst)
- Spinning Disk Microscopy (N de Ruijter)
- TIRFM (Johannes Hohlbein)
- Multi-photon microscopy
- Light sheet microscopy (SPIM)

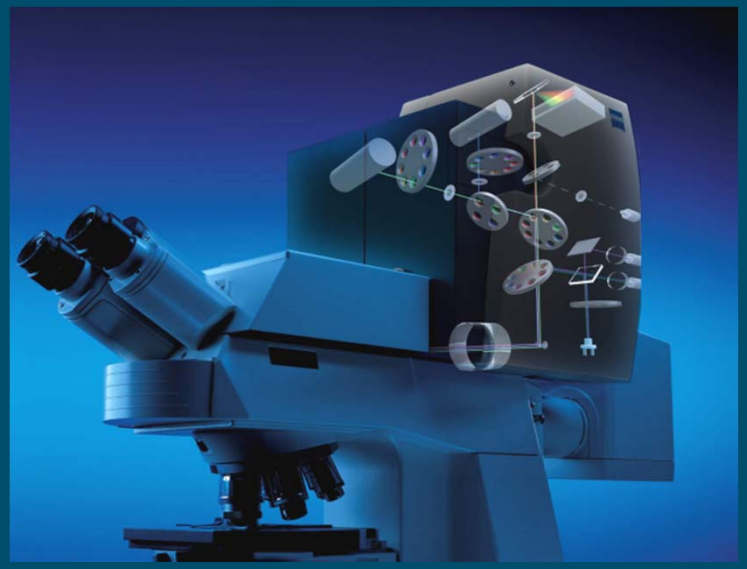
- Mathematical: Image Deconvolution (Image J, Matlab)

Confocal Principle

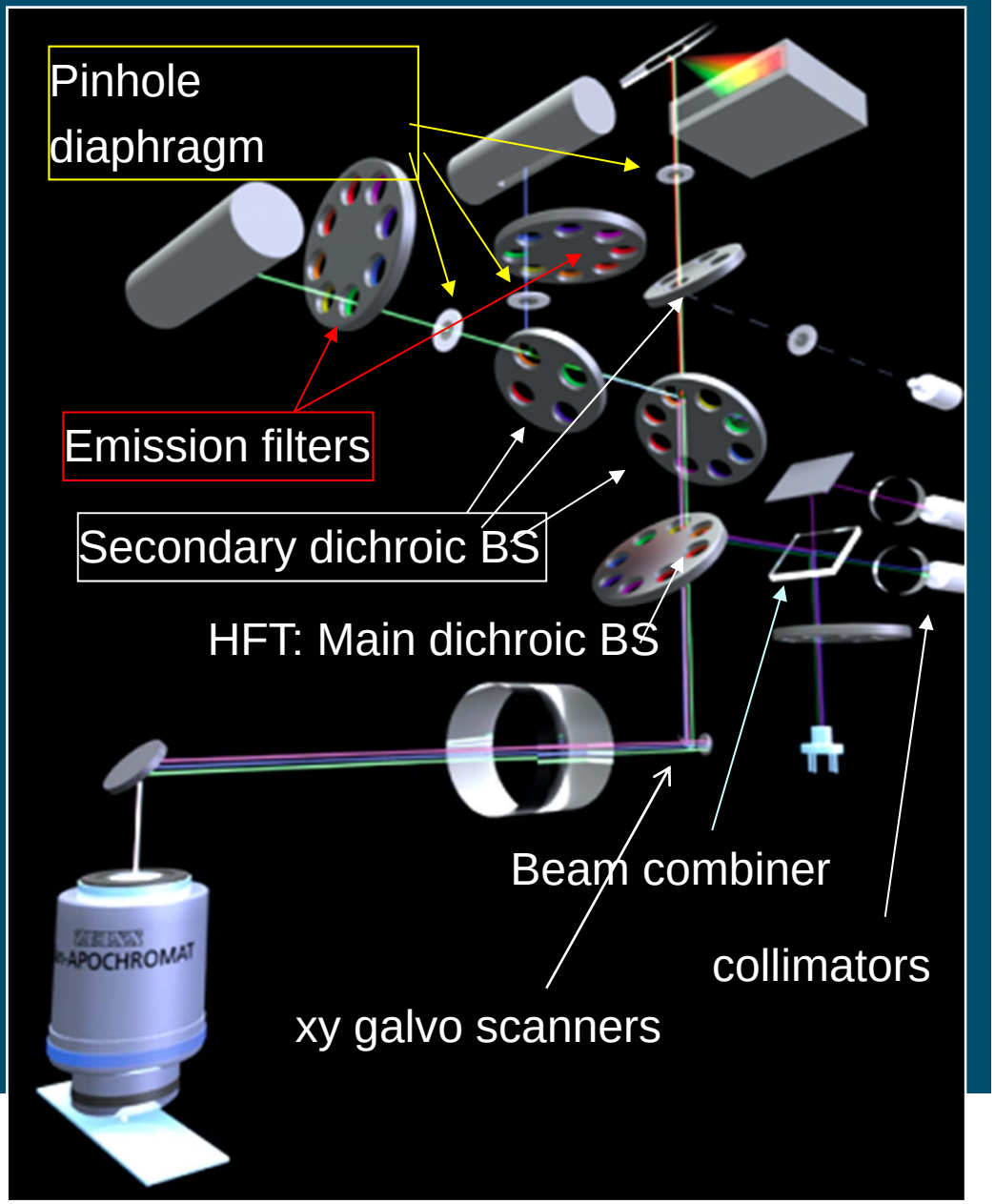


Blocking out of focus light

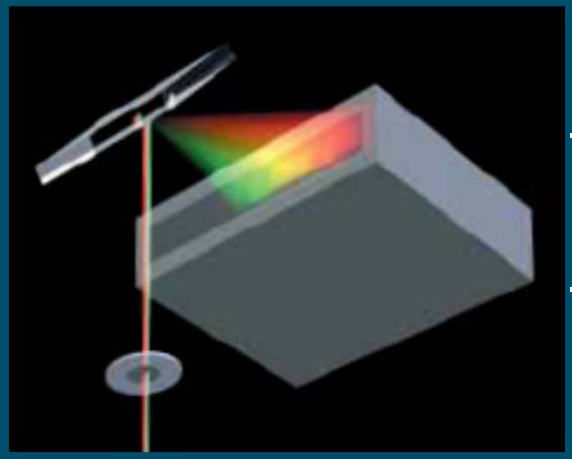
Configuration for multiple color detection



Beam path in the scan head of a point scanning LSCM - META



Prevent signal bleed through by META detector



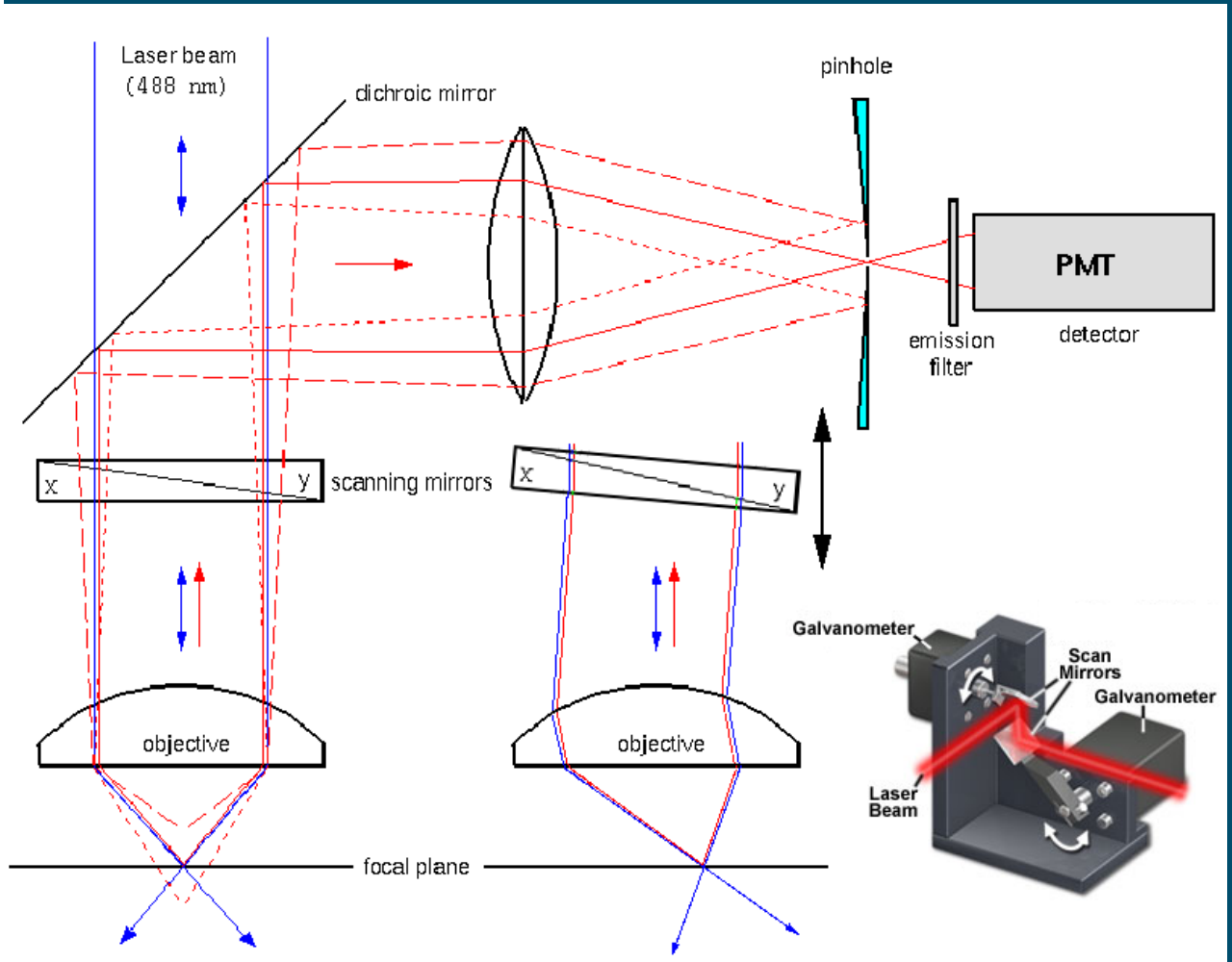
-Diffraction grating splits emission spectrum over set of 32 channels (PMTs)

-The spectral signature of each pixel is acquired

> Imaging with spectral unmixing :

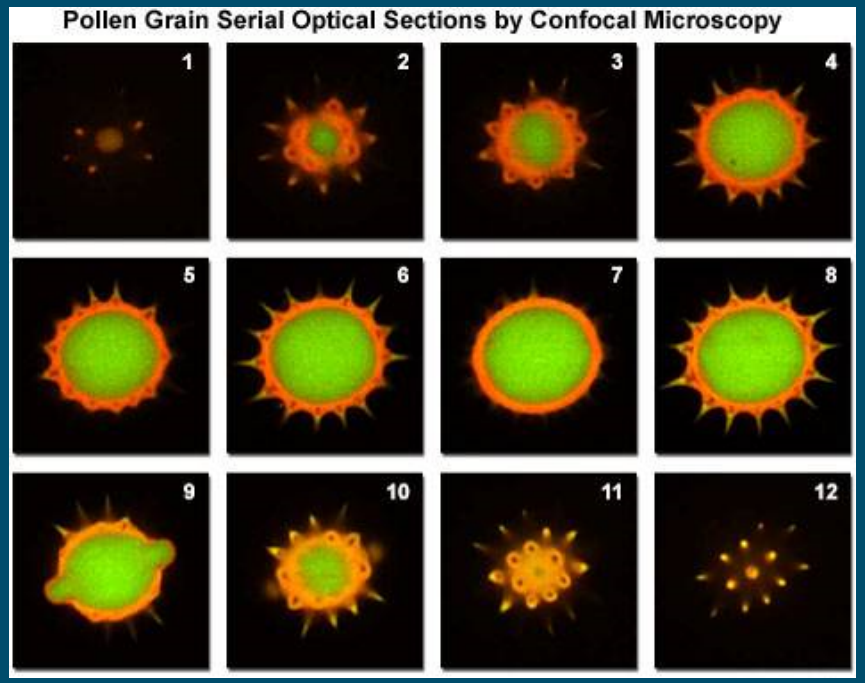
- is rapid and increases sensitivity
- is specific and allows to separate overlapping EM spectra
- is more reliable than filter sets and band pass acquisition
- allows high speed multicolor imaging
- disadvantage: only one pinhole/gain setting for all emissions

Confocal laser scanning microscopy (galvo mirrors)

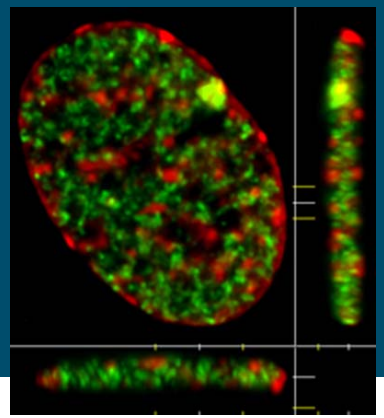


- With a pinhole the confocal LSM can make optical sections of a sample (xy plane)
- A z-serie of optical sections is used to reconstruct a sharp 3D image.

Optical sectioning with CLSM



BY-2 tobacco suspension cell expressing GFP-ER (zs)

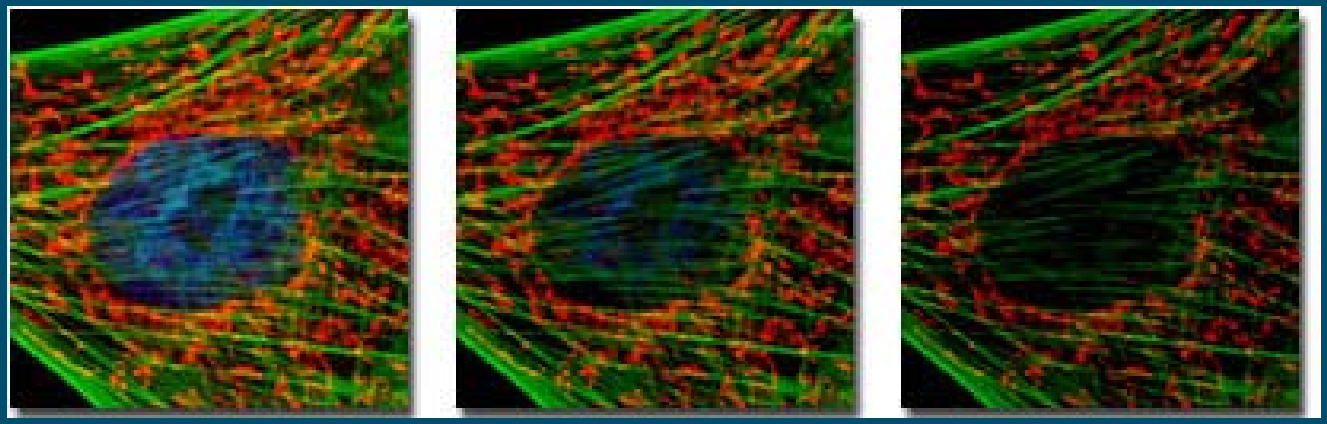


Orthogonal view;
more depth info in (flat) nucleus

Confocal Laser Scanning Microscopy

- a focused laser beam scans (xy) spots on specimen
- optical slices are collected over time (ts) or (and) in subsequent different focal planes (zs) in various channels (colors)
- computer reconstructs a 3-dimensional image by compiling optical slices
- bleaching of out of focus fluorophore however, can still be strong; a pinhole is only present in the emission path

Photo bleaching - long term imaging

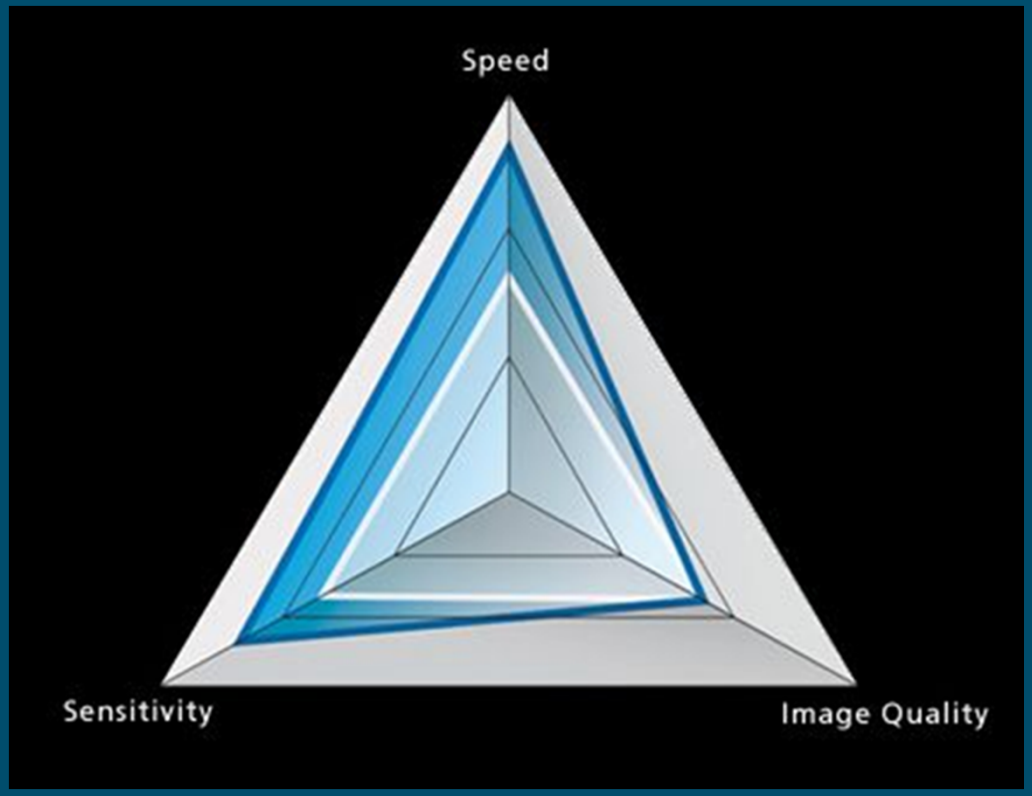


Excitation intensity usually decays when imaging a sample for longer time because fluorophores are irreversibly photo bleached. Some fluorophores are more stable than others.

Prevent bleaching: low excitation intensity, short exposure....

> Find imaging parameters to obtain bright, sharp fluorescent pictures with minimal bleaching.

Imaging: balancing sensitivity, speed, resolution

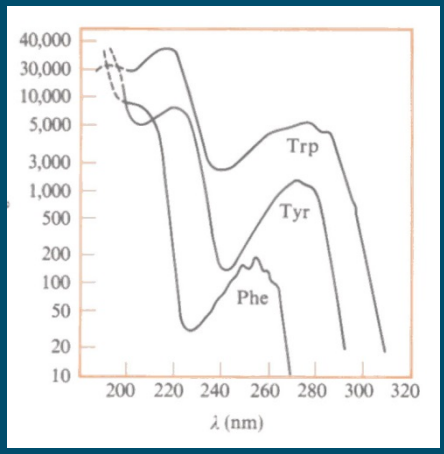


Signal / noise ratio relates to

- scanning speed
- pixel density
- pinhole size
- gain

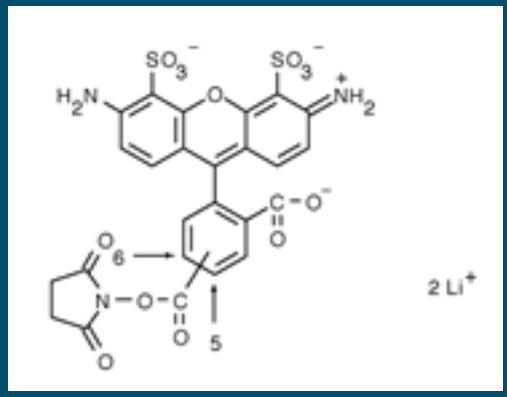
Fluorescent Labels

- Intrinsic fluorophore
  - e.g. Tryptophan, chlorofyl, lignin



amino acids

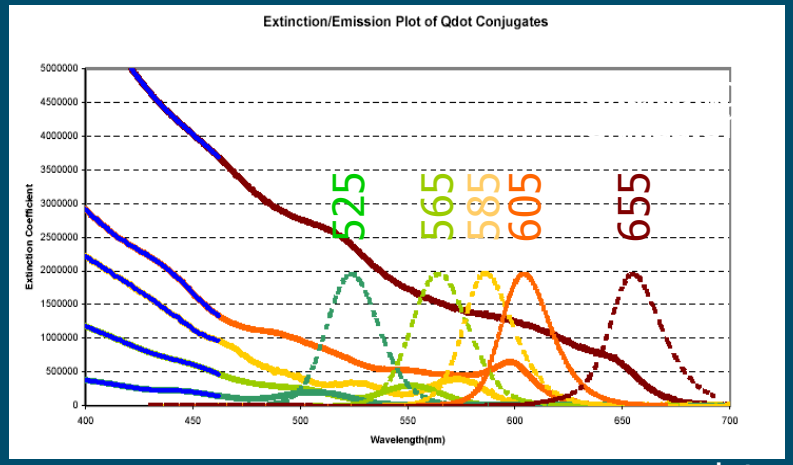
- Chemical dye
  - Alexa dyes, Cy dyes
  - Immuno labels
  - Covalent binding



Alexa 488 succimidyl ester

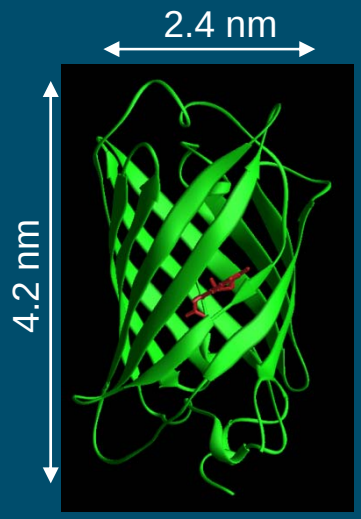
Fluorescent Label

- ✓ Quantum dots
  - + photo stable
  - + high quantum yield
  - + nano particles

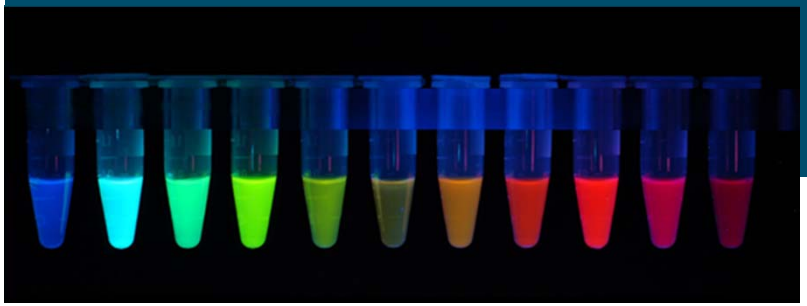


www.qdots.com

- ✓ Green fluorescent protein
  - + ‘genetic’ labeling
  - + well protected from environment
  - - size



Ormő et al. *Science* (1996), **273**, 1392



Various FL probes allow quantitative functional imaging

(seeing molecular dynamics)

- Fluorescence ratio imaging (internal control)
- Fluorescence Recovery After Photobleaching (dynamics)
- Photo activation
- Photo conversion
- Uncaging
- Bimolecular complementation
- More: Biochemistry
  - FCS – study mobility of molecules
  - FRET- follow molecular interactions

Imaging centres within WUR

NMR: Nuclear Magnetic Resonance Imaging (Transitorium -Dreijen)
WEMC: Wageningen Electron Microscopy Centre (Radix West-campus)
WLMC: Wageningen Light Microscopy Centre (Radix West-campus)
MSC: Micro-Spectroscopy Centre (Transitorium -Dreijen)

We share forces and merge nto one virtual centre called WISH:
Wageningen Imaging & Spectroscopy Hub

Currently in the process of being officially recognized / accepted as a NL-Bio Imaging Centre and Euro Bio Imaging Centre for plant imaging.....

Aims: more options

- to further maintain /develop imaging facilities at WUR
- to facilitate / assist you and foreign visiting scientists
- for industry/institutes to access up-to-date equipment

Where to go?

		Cell Biology (PSG)-WLMC-Radix	Biochemistry (AFSG)-MSC- Dreijen	HMI-ASG (Karczewski/Schipper)
Macro and FL-stereo		X		
DIC/PhC/BF/HMC (unstained-sterile)		X		
Micro manipulation (Narashigi) and LM		X		
Polarization (quantified)		X		
Fluorescence -wide field - multicolor		X	X	X
Confocal - imaging plant structure-morphology		X		X
Confocal - imaging structure-morphology poor signals	spinning disk/ MM-CLSM	X	X	
Confocal - imaging structure-morphology dynamics/ FRAP	spinning disk	X		
Confocal – temperature and CO2 control on stage	spinning disk	X		
Confocal - ion fluxes dynamics		X		
Confocal – combined with Optical tweezers		X		
Confocal - colocalization	spectral unmixing	X	X	
Confocal - colocalization quantified		X	X	
Confocal - protein fast dynamics, quantified	MM-CLSM		X	
Confocal - protein interactions localized		X	X	
Confocal - protein interactions, quantified, FCS	MM-CLSM		X	
Confocal - deep into tissue-/ multi-photon			X	
Structural properties of proteins-distance /FRET	MM-CLSM		X	
Structural properties of proteins-folding/dynamics/FLIM	MM-CLSM		X	
TIRF - FL events at the plasma membrane/ in vitro assays	sm-TIRF	X	X	
High througput/content	BD pathway			X
Additional support				
Embeddings and microtomy		X		
Support for protein purification and labeling			X	
Range of available life probes for testing		X	X	
Options for optimal mounting for prolonged life imaging		X		

End intro

Questions?