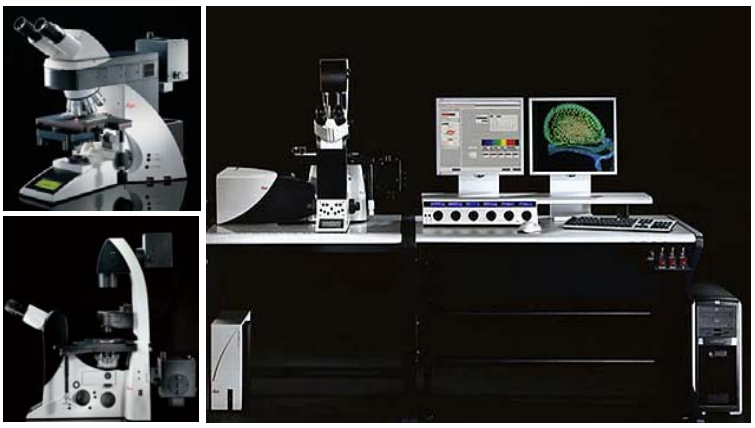
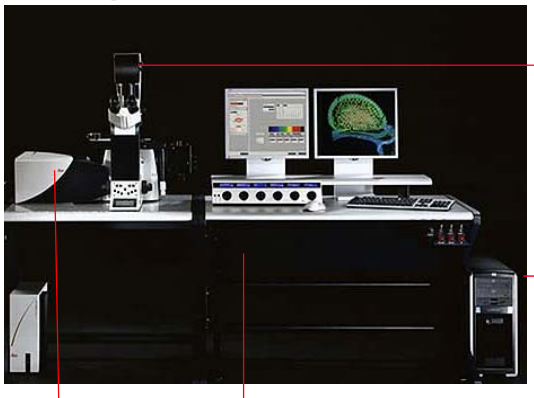
Leica TCS SP5 共軛焦顯微鏡的原理及應用



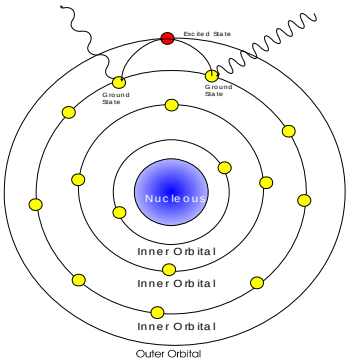
美嘉儀器股份有限公司
產品技術專員 劉思嫻
www.major.com.tw

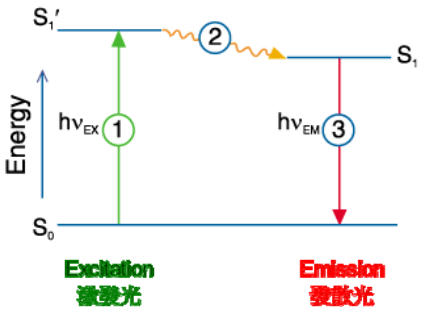
Leica TCS SP5

Main configuration :



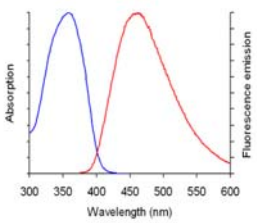
What is Fluorescence ?



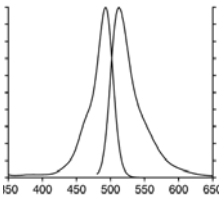


Fluorochrome

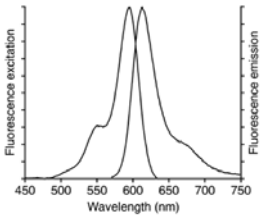
DAPI

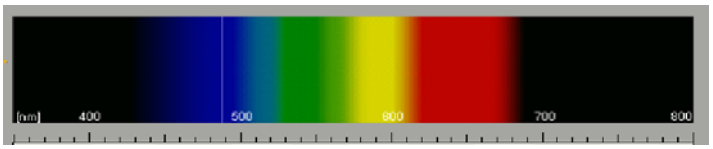


Fluorescein



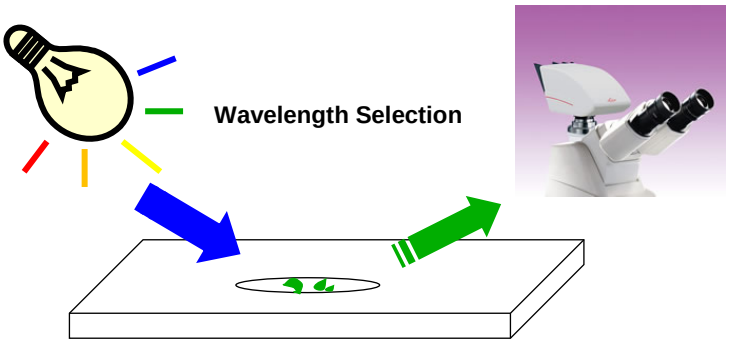
Texas Red





Illumination System (Excitation)

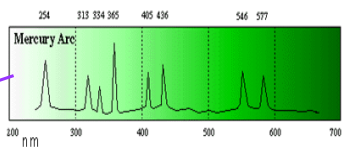
Detection System (Emission)



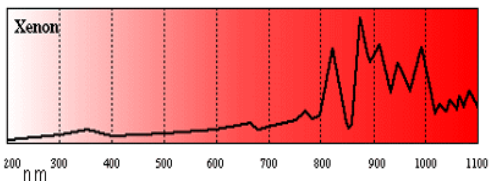
Conventional Fluorescence Microscope

Illumination System (Excitation)

HBO



XBO



Conventional Fluorescence Microscope

Wavelength Selection

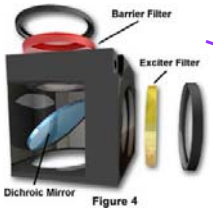
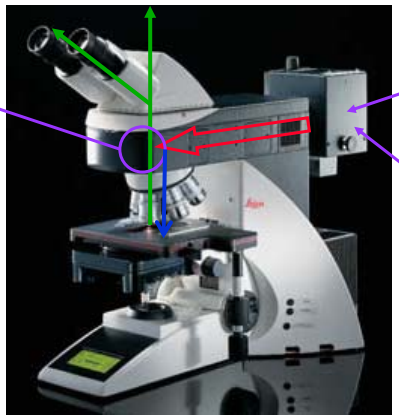
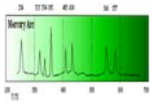


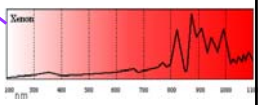
Figure 4



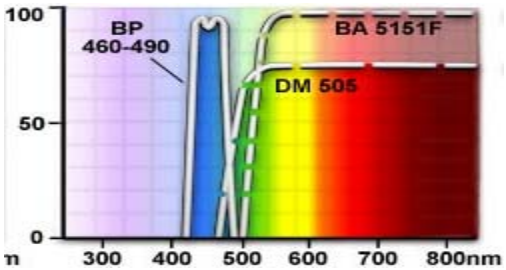
HBO

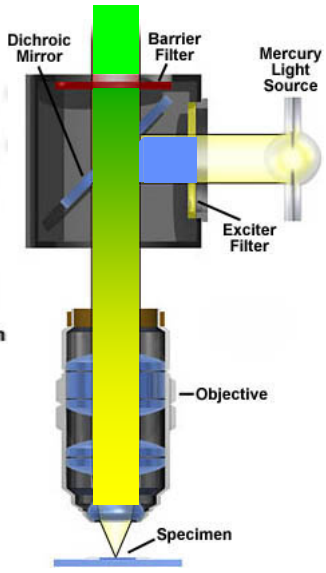


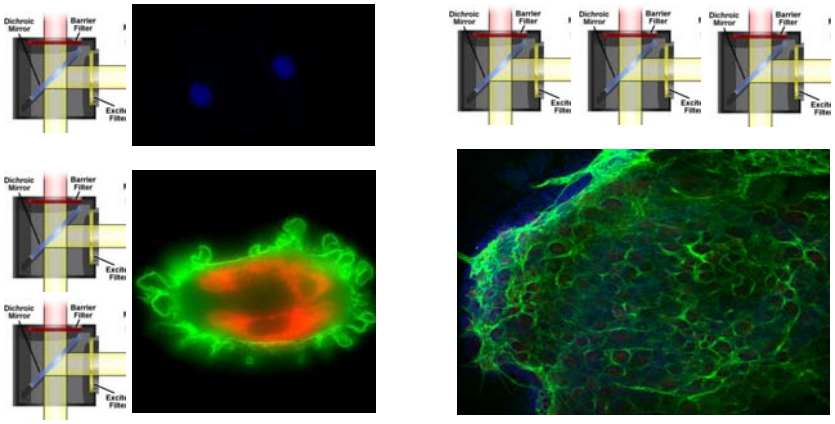
XBO



Conventional Fluorescence Microscope



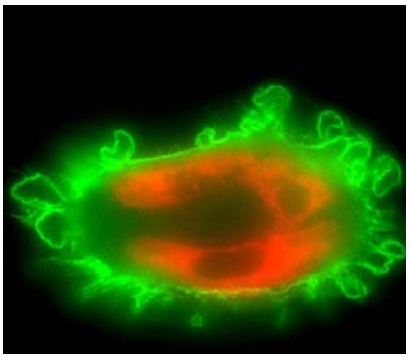




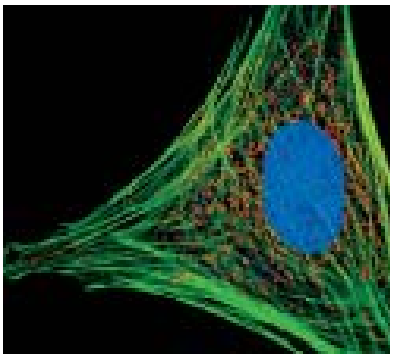
One cube for one dye

Imgae Quality ?

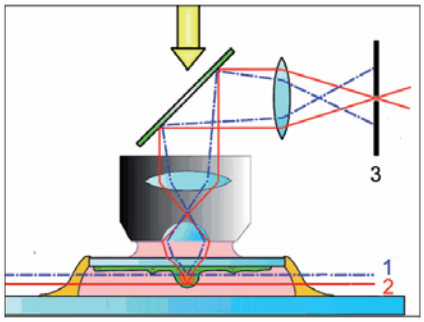
Conventional Microscope

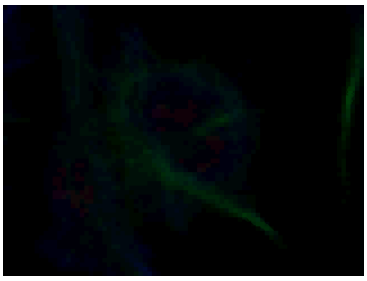


Confocal Microscope



What is Confocal ?



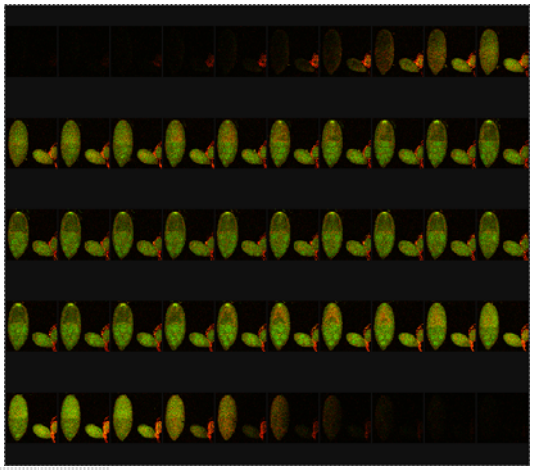


Why confocal microscopy ?

Optical sectioning:

Specimen is monitored slice by slice (3D-resolution)

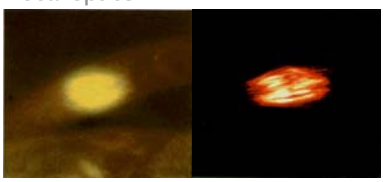
Each slice produces a sharp image by confocal optics



Why confocal microscopy ?

Optical sectioning:
Specimen is monitored slice by slice (3D-resolution)
Each slice produces a sharp image by confocal optics

Improved resolution power (PSF) :
lateral resolution improved

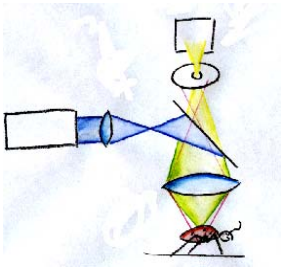


Improved contrast:
Rasterizing the specimen, stray light due to scattering is suppressed

Multi-dimentional acquisition with digital image processing
X-Y-Z-T
2D: xy, xz, xt
3D: xyz, xyt....
4D: xyzt.....

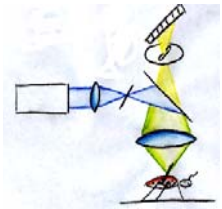
New application, FRAP, FLIM, FRET, Cage, Bio-Mapping .....

Different Techniques of Confocal Microscope



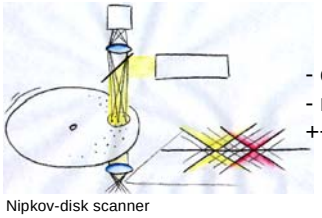
point confocal

++ contrast
++
resolution
- speed



- contrast
- resolution
+ speed

l slit confocal



- contrast
- resolution
++ speed

Nipkov-disk scanner

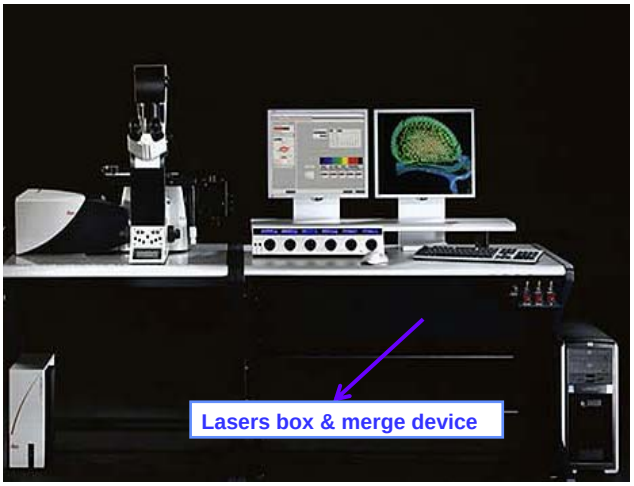
Only single point confocal has best resolution & contrast
Leica TCS NT/SP2/SP5 Confocal System is a single point confocal

Confocal System

Main configuration :

1. **Illumination (Excitation) System**
2. **Confocal Scanning & Detection (Emission) System**
3. **Microscope System**
4. **Data Control System & Application Software**
5. **Sample preparation**

Ilumination System



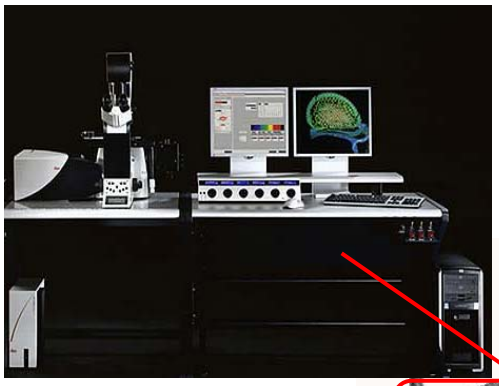
Visible Lasers

	Type	Wavelength	Fluorochrome
Violet	DPSS, 8mW	430 nm	Alexa 430, CFP..
	HeCd, 20mW	442 nm	
Blue	Argon, 100mW	458, 476, 488, 496*, 514 nm	Alexa 488, Cy 2, FITC, eGFP, YFP…
Green	HeNe, 1mW	543 nm	Alexa 546, 555, 568, Cy3, TRITC, DsRed..
Light Green	DPSS, 10mW	561 nm	
	Kr, 25mW	568 nm	
Orange*	HeNe, 2.5mW	594 nm	Alexa 594, Texas Red, Mito Tracker Orange/Red….
Red	HeNe, 10mW	633 nm	Alexa 633, Cy5..
Multi λ	Ar-Kr, 75mW	488, 568, 647 nm	Blue/Green/Red

UV – 405 nm Lasers

	Type	Wavelength	Fluorochrome
UV	Argon, 50mW	351, 364 nm	DAPI, Hoechst...
➲ 2005 Violet	Diode, 50mW	405 nm	DAPI, Hoechst, BFP, CFP...

Ilumination System



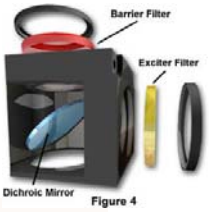
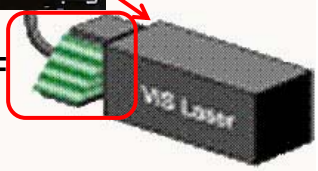


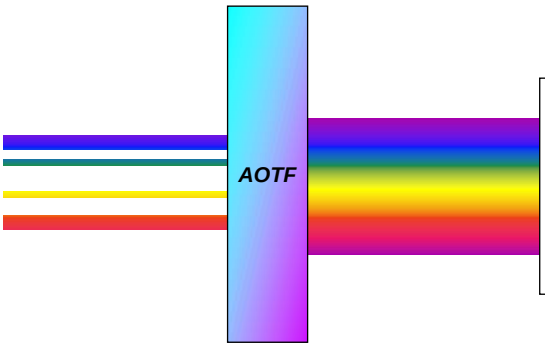
Figure 4

AOTF



AOTF - Variable Excitation Filter

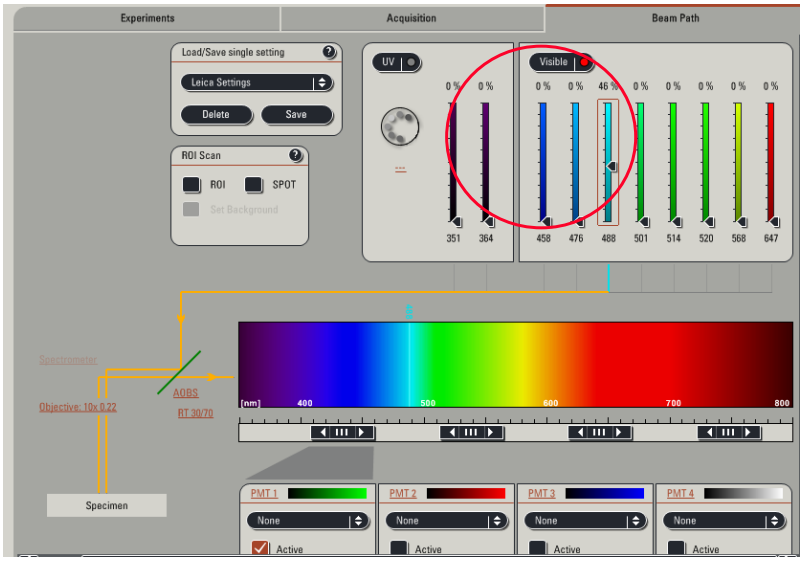
Acousto Optical Tunable Filter



Light source:
Multiline Lasers
Multiple Lasers,
optically merged

- ✓ Independent line selection
- ✓ Independent intensity control

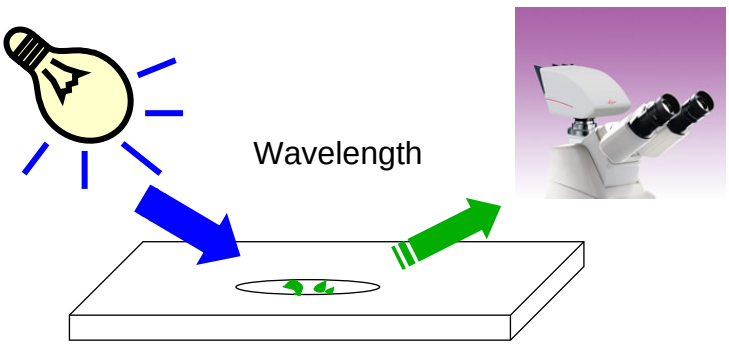
AOTF – Software Control



Detection Method

Illumination System
(Excitation)

Detection System
(Emission)



Detection Method

- PMT – PhotoMultiplier Tubes

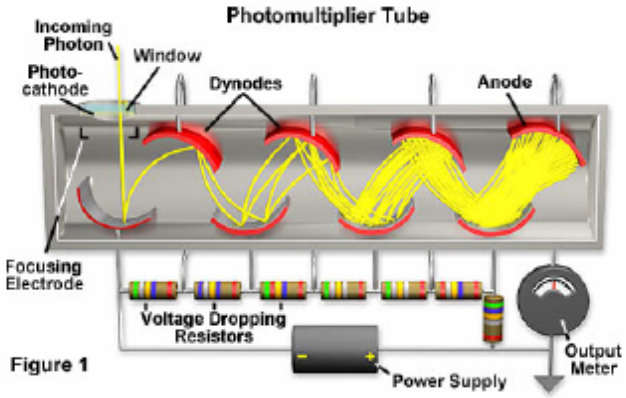
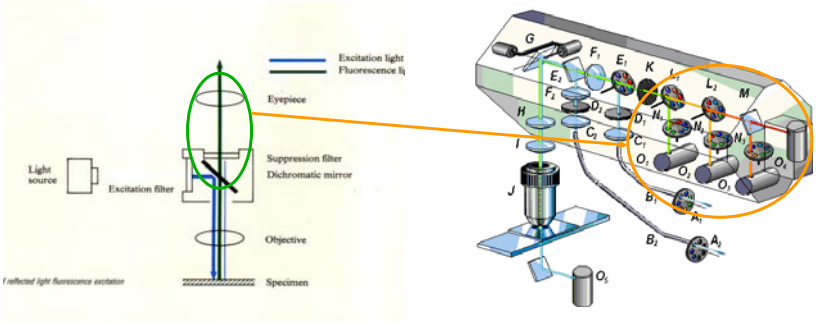


Figure 1

Detection Method

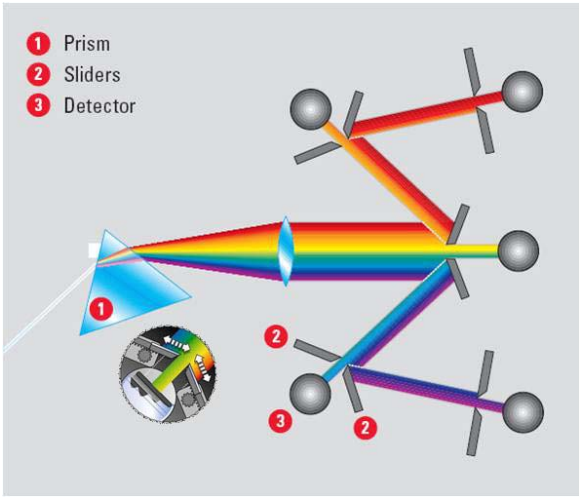
- **Filter**
- **Spectral (Slit)**

Detection System - Filter Base -



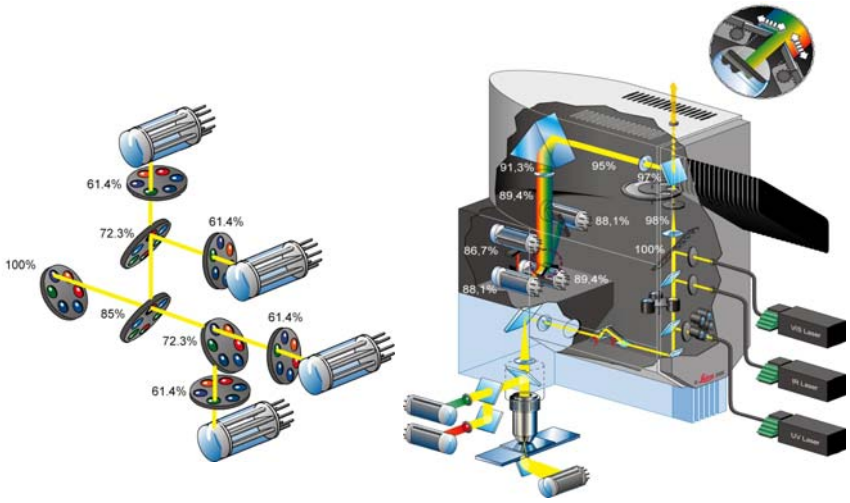
- 每一個螢光偵測器前都有一組Dichromatic mirror及Suppression filter
- 使用者需熟知所使用螢光染劑的特性, 以選擇適當的螢光濾鏡
- 螢光濾鏡更換的速度永遠跟不上螢光色原變換的速度
- 早期的LEICA (TCS NT)與現今其他廠家的共軛焦系統仍採用此裝置

Spectral Imaging Detector SP

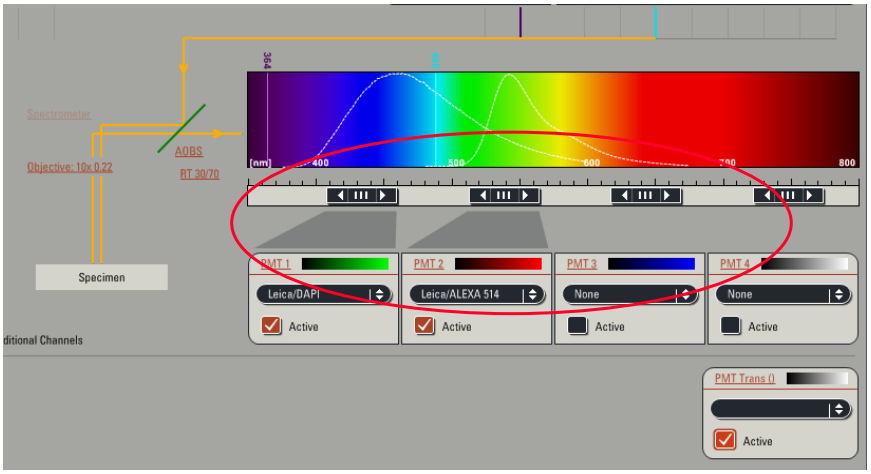


Detection System

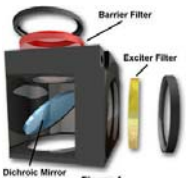
Filter Base vs. Spectral Base

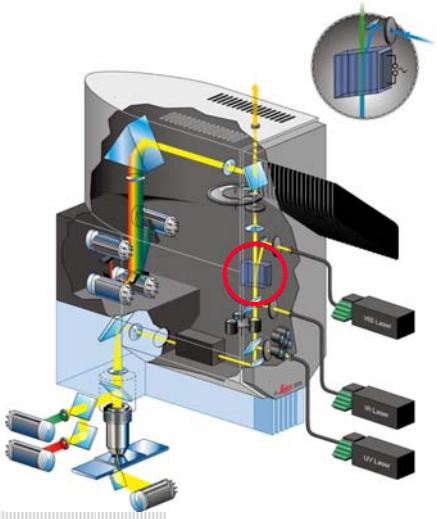


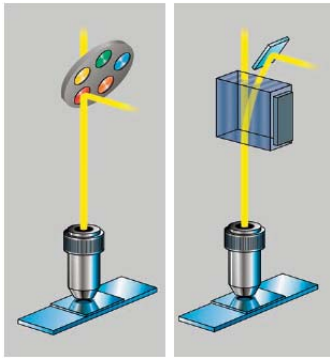
Spectral Based Detector



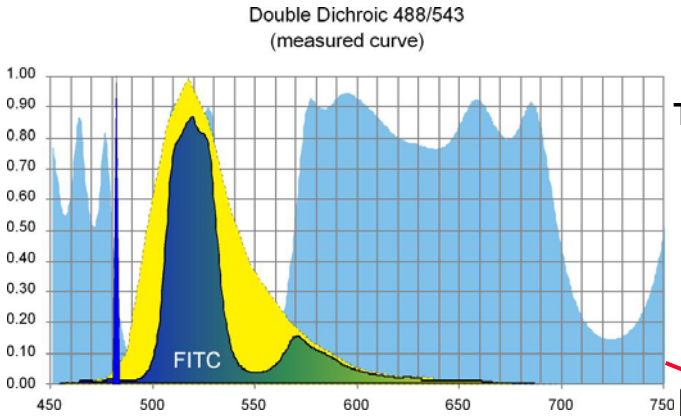
Leica 2002
Beam Splitting Control: AOBS
(Acousto Optical Beam Splitter)



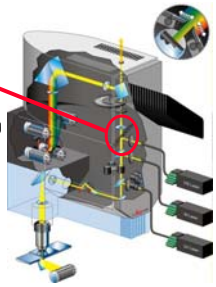




Beam splitter limitations

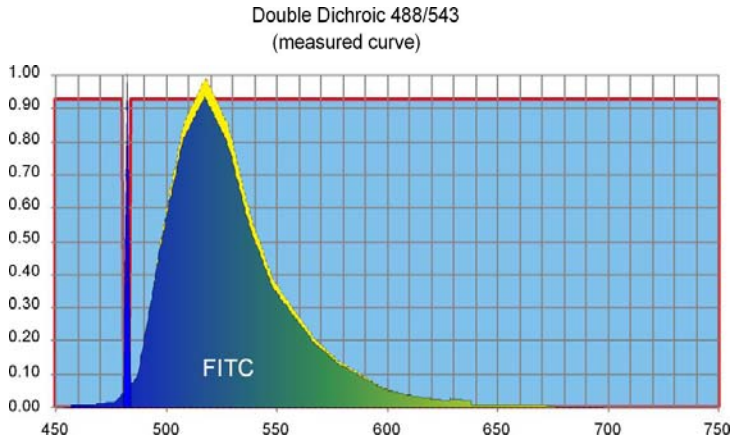


Transmission

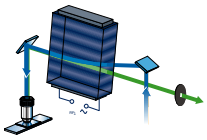


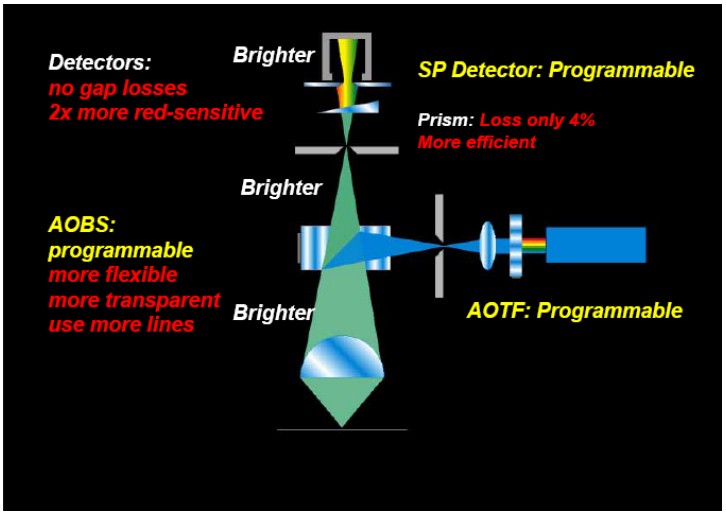
- **No sharp bands**
- **Transmission holes**
- **Fixed characteristics**

AOBS: More efficient



- ✓ Perfect selectivity (0,6-2 nm bandwidth)
- ✓ More transparent than beam splitters
- ✓ More "room" to detect fluorescence





What Do We Have?

Upright Confocal System – TCS SP5

DM 5000B CS

Manual Z-focus, nosepiece

Automatic BF/DIC

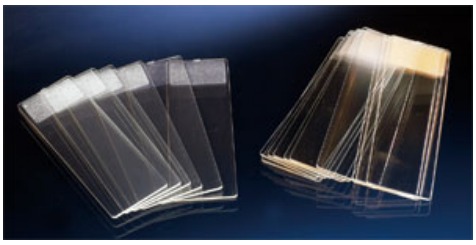
Filter cubes: A for UV excitation

I3 for Blue excitation

N2.1 for Green Excitation

Vis/ Scan

Microscopes & Samples



Objectives

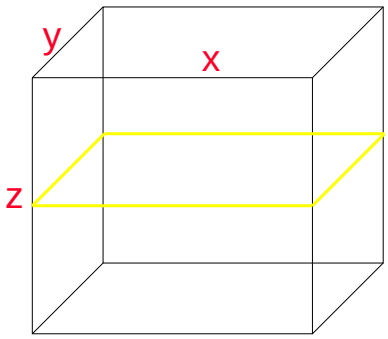
Objectives	Dry/Imm	DIC	Sample Types
HCX PL APO 10x/0.40	Dry		0.17 mm cover glass
HCX PLAN APO 20x/0.7 Corr λ - blue	Imm		--
HCX PLAN APO 40x/1.25 -0.75 λ - blue	Oil	√	0.17 mm cover glass
HCX PL APO 63x/1.40-0.60 λ-blue CS	Oil	√	0.17 mm cover glass
HCX PL APO 100x/1.40 Oil CS	Oil	√	0.17 mm cover glass

Z Section

Galvo Z Stage

Application

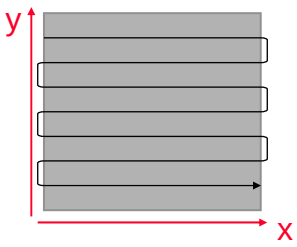
xy



Acquisition of a single frames

Beam is scanned
in x and y direction
in the focal plane by
moving scan mirror

Scanning time depending on
scan format (number of lines)



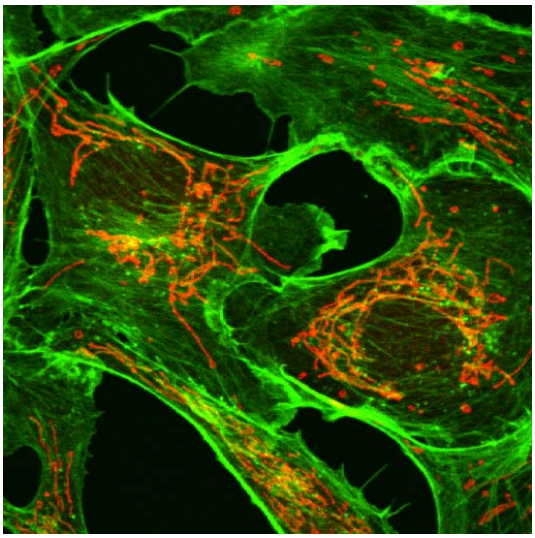
Major Instruments Co., Ltd. Taiwan

xy scanning

Multidimensional
Confocal Imaging

Leica MICROSYSTEMS

- Sample overview
- Colocalization studies
- Resolution-enhanced, high contrast images

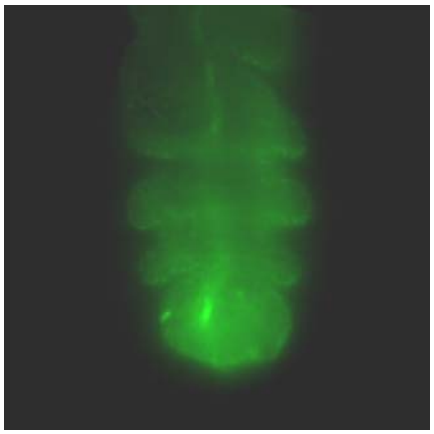


Endothelial cells
FITC (Actin)
Mito-Tracker

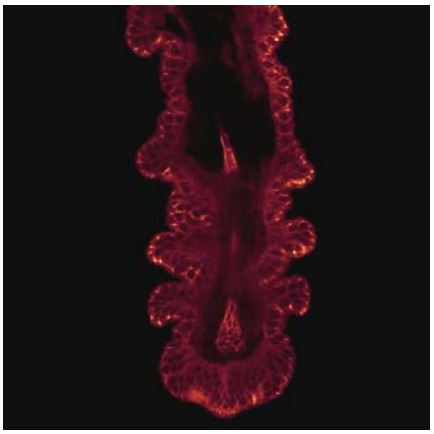
xy scanning

Multidimensional
Confocal Imaging

Leica MICROSYSTEMS



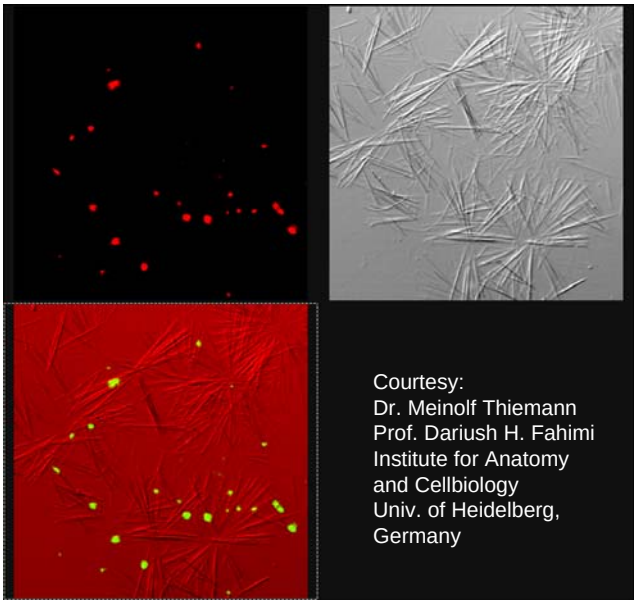
Drosophila leg, FITC, non-confocal



confocal

Multidimensional
Confocal Imaging

xy scanning



Courtesy:
Dr. Meinolf Thiemann
Prof. Dariush H. Fahimi
Institute for Anatomy
and Cellbiology
Univ. of Heidelberg,
Germany

Peroxisomes,
GFP, 488 nm

Microtubules,
Transmission

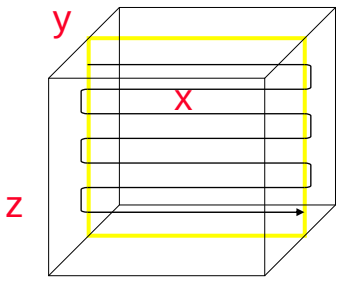
Interaction of
peroxisomes with
Microtubules

Eur. J. Biochem. 2000

Multidimensional
Confocal Imaging

xz

Beam is scanned in x-direction
Sample is moved in z (z-stage)



Z resolution depends on axial resolution of objective, generally 2x less than in xy
xy: 180 nm, z: 360 nm

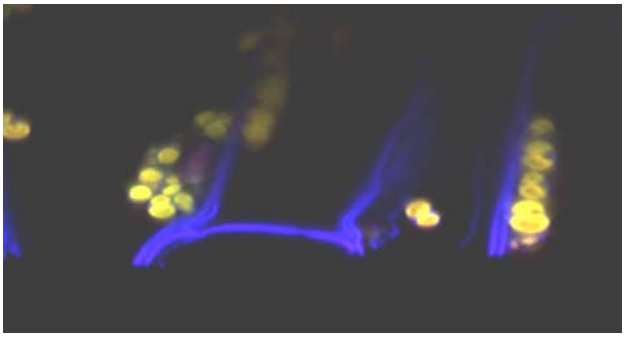
- Orientation of sample
- Spatial relations between structures in z
- Polarized cells

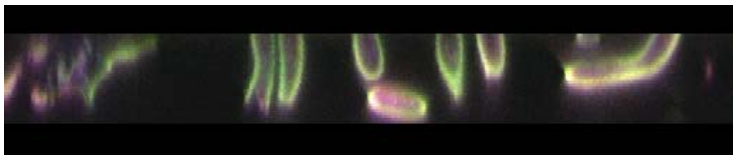
xz scanning

- Up to 20 frames per second with the Leica TCS SP2!

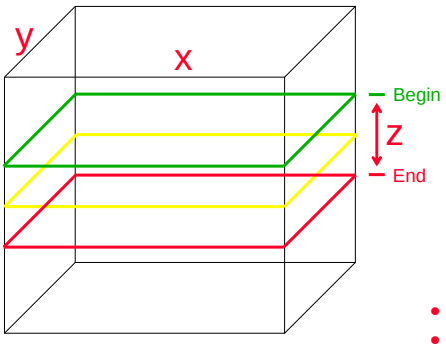
Convallaria

Starch grain
Cell wall





xyz

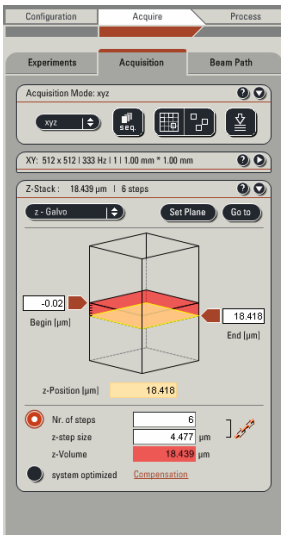


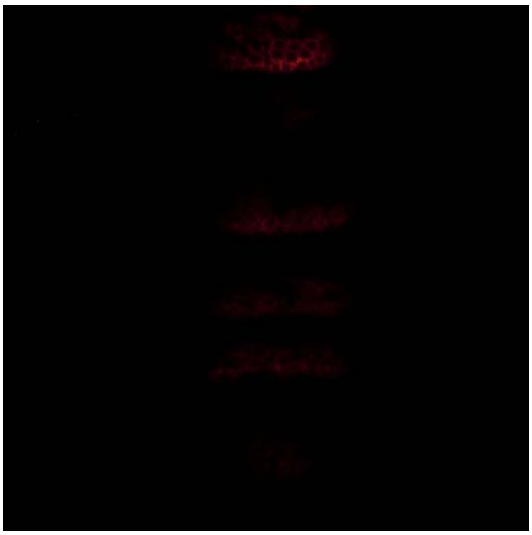
Beam is scanned in x and y direction and sample is moved in z via galvo stage or electronic focus of microscope

- Developmental Biology
- Neuroscience

- Optical sectioning,
- 3D stacks
- 3D projections
- 3D Animations
- Structural information from large focal depth – just depending on the stack size!

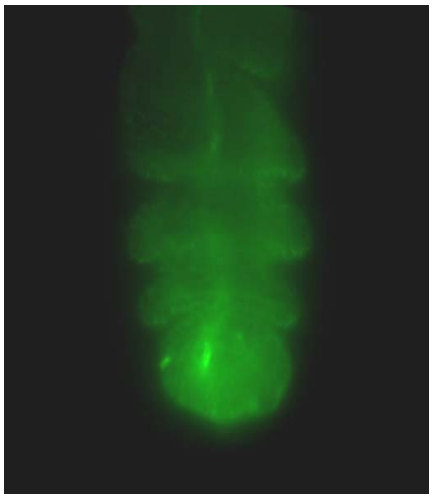
XYZ -- Software



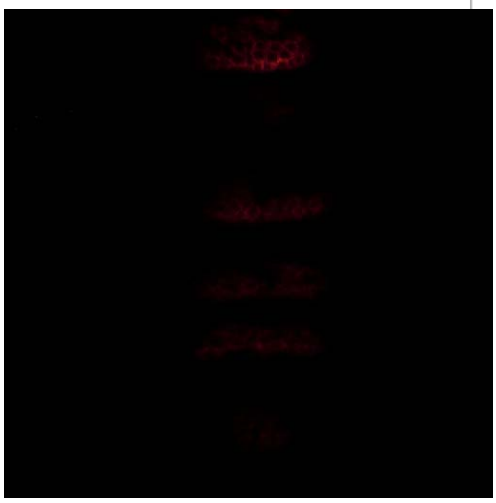


Multidimensional
Confocal Imaging

xyz scanning



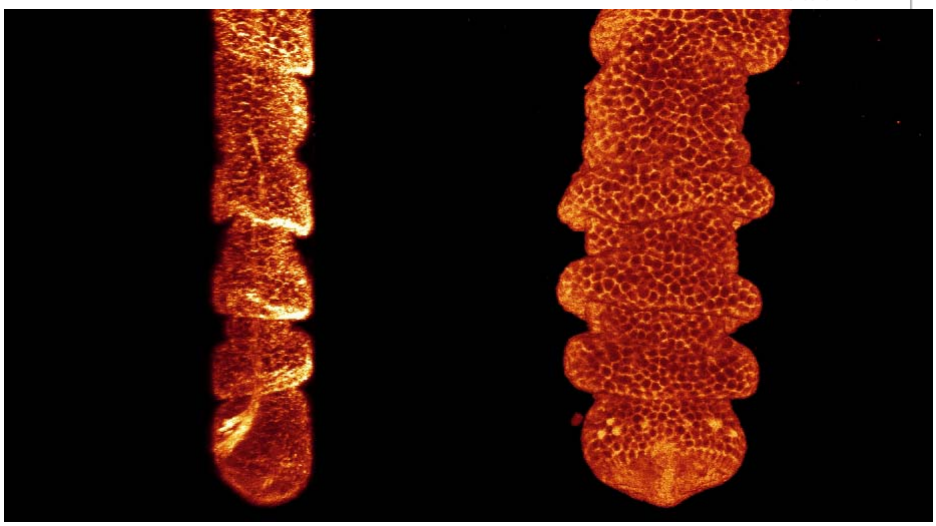
Drosophila leg, FITC, non-confocal



confocal

Multidimensional
Confocal Imaging

xyz projections: different algorithms



Drosophila leg, FITC, projection

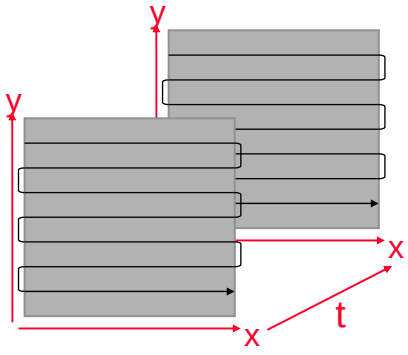
Surface rendering

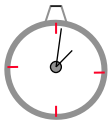
Multidimensional
Confocal Imaging

xyt

beam scans sample in x and y direction over time (defineable)

Time sequences

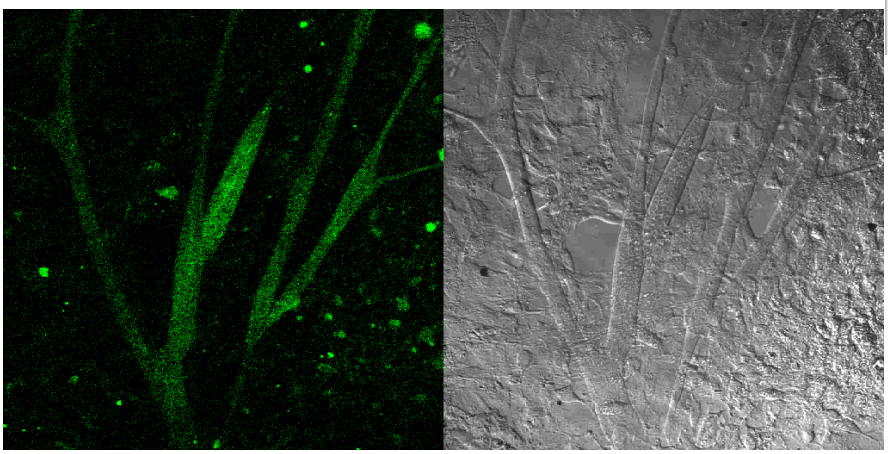




Dynamics
life cell research

- Protein/organelle transport using FRAP
- Uncaging
- Physiological activity (ion measurements with ratiometric dyes like Indo-1, Fura-2)
  ROI based quantification

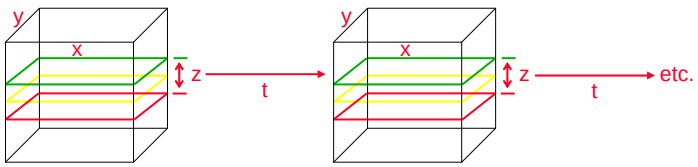
Application



CEAGrenoble myotubes.avi
Time lapse movie of calcium waves in cardio myocytes in fluorescence and transmission. Label in fluorescence was Fluo-3
Courtesy: Mme Marty et Mr Grunwald du CEA de Grenoble
Laboratoire des canaux ioniques et signalisation, 38054 GRENOBLE Cedex 9

Multidimensional
Confocal Imaging

xyzt



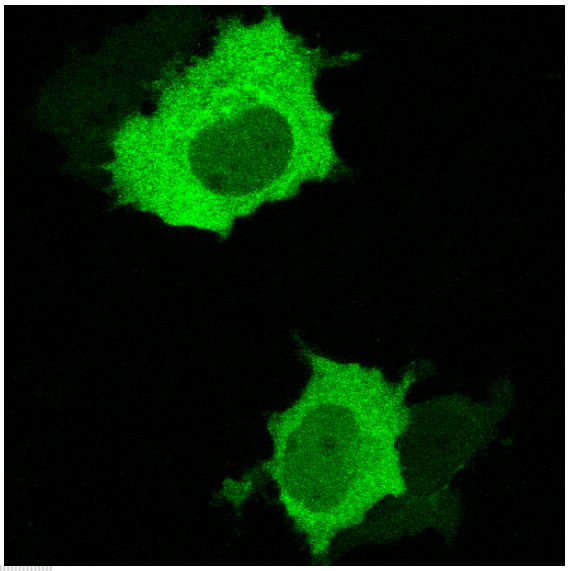
Structural information out of
all dimensions in space and time

Dynamic processes — Sets 3D image stacks acquired in time intervals

Developmental Biology — Powerful configurable timelapse feature

Multidimensional
Confocal Imaging

xyzt scanning



Thank you !