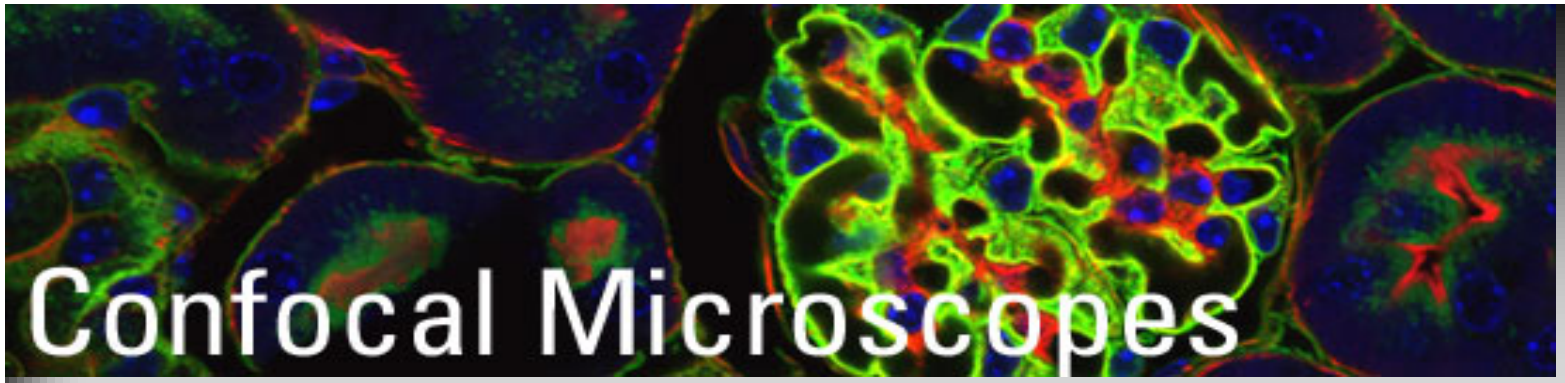
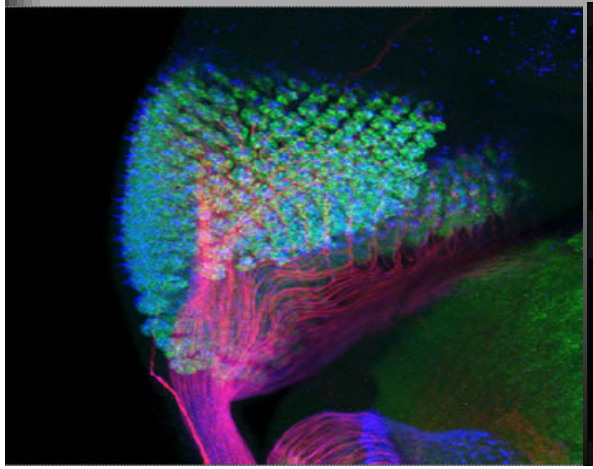
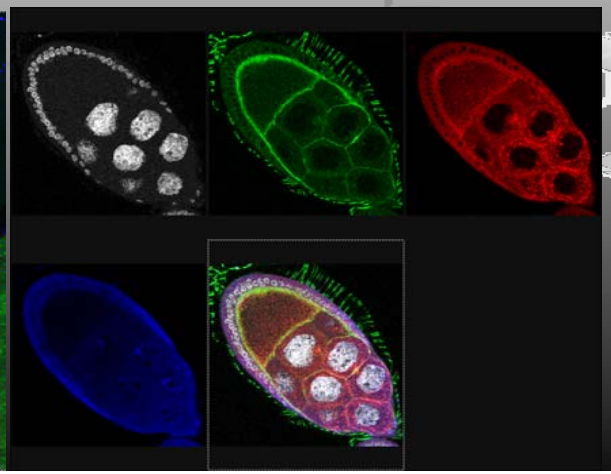
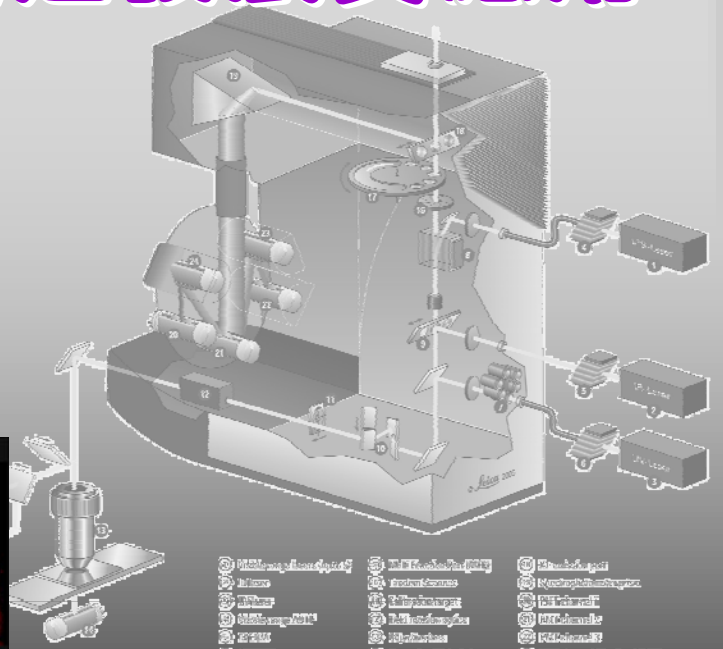


雷射掃描共軛焦顯微鏡之發展與應用

LEICA TCS SP5 AOBS







美嘉儀器股份有限公司
共軛焦影像技術部 廖世懿
www.major.com.tw
dick.liao@msa.hinet.net

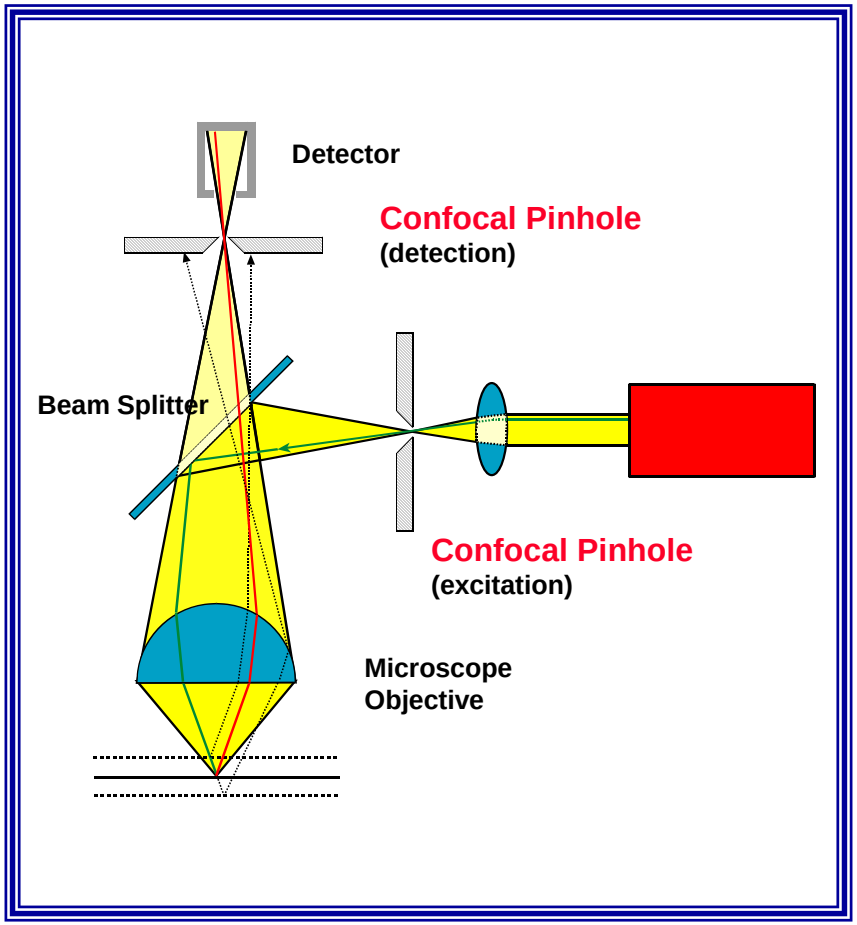
Confocal Microscopy

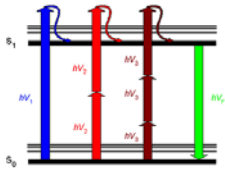
- Confocal Laser Scanning Microscope -

- What is Confocal?
- Confocal Microscope Development:
  - From filter to *AOTF*
  - From filter detector to *Spectral detector*
  - From dichroic to *AOBS*
  - From Multi-line laser to *White Light Laser*
- New Generation Confocal Microscope
  - Uniting two worlds: high resolution and high speed
  - LEICA TCS SP5
- Application

Leica MICROSYSTEMS

What is Confocal ?





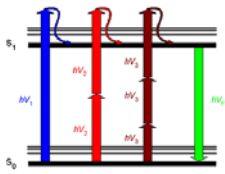
Fluorescence microscope vs Confocal system

Leica
MICROSYSTEMS

What's Different ?

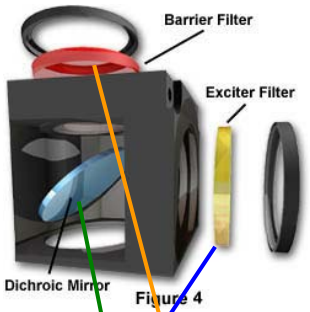
Fluorescence microscope

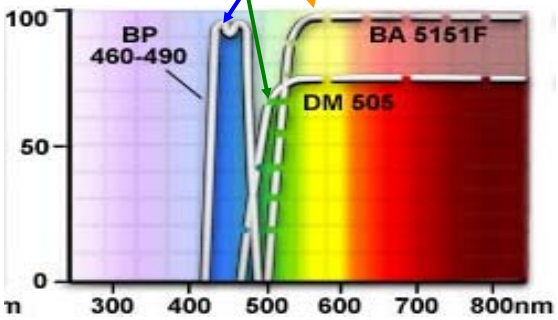
Confocal system

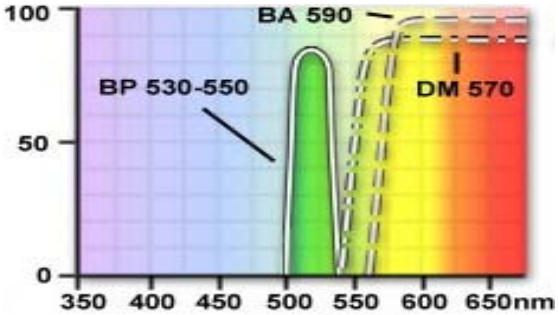


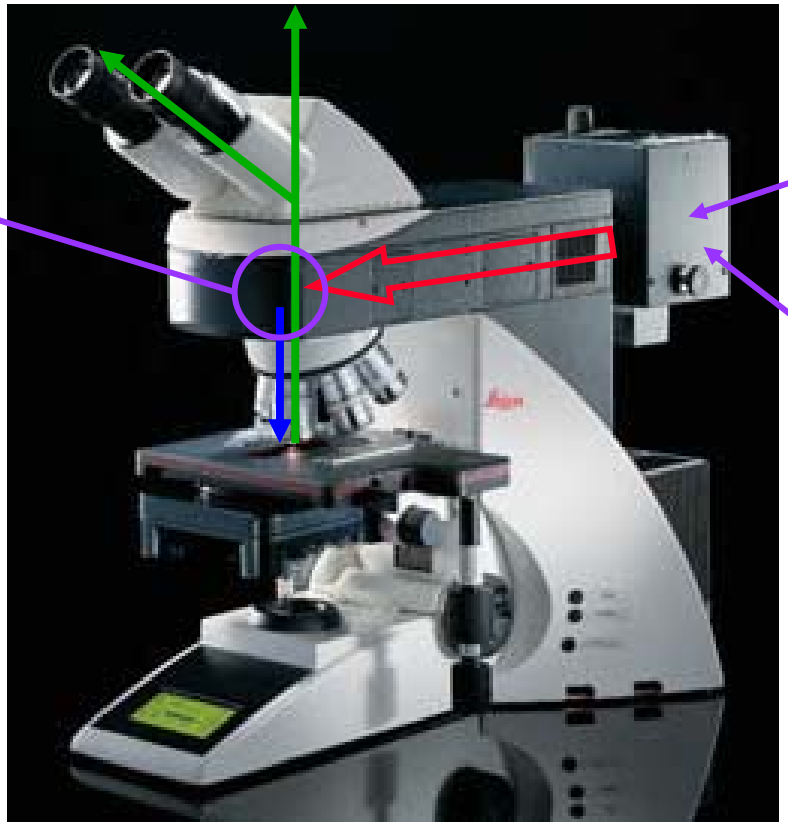
LEICA Confocal Microscopy

Leica
MICROSYSTEMS

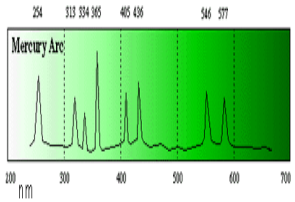




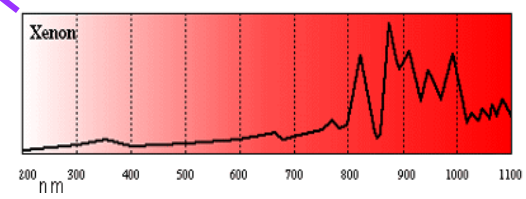




HBO

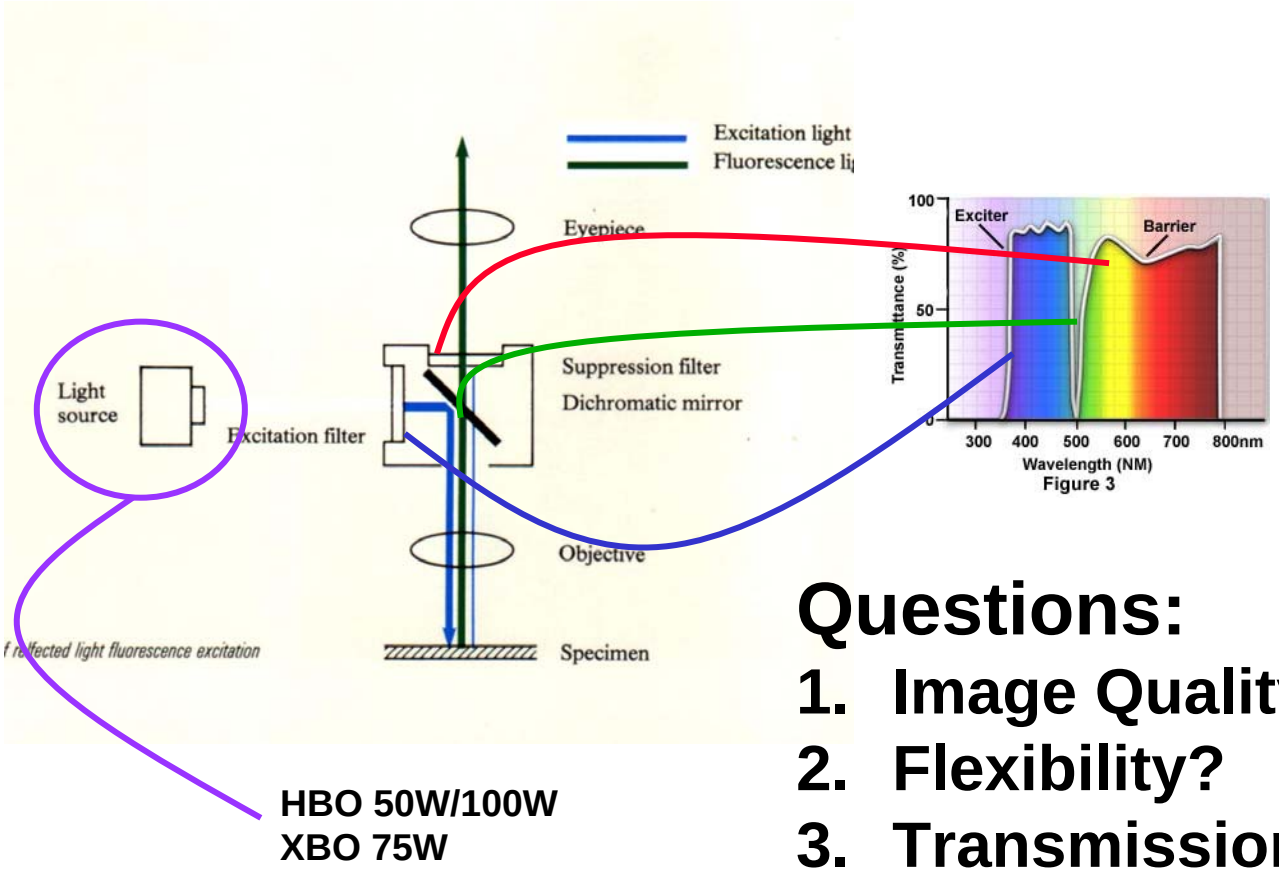


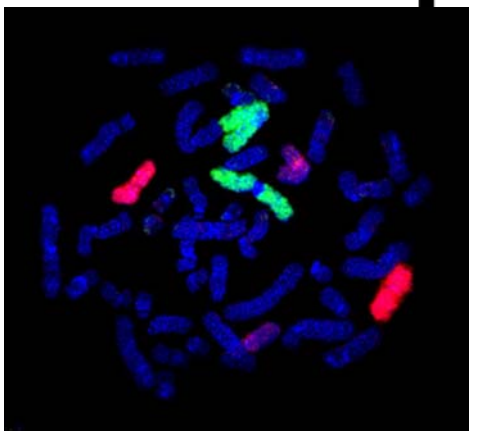
XBO

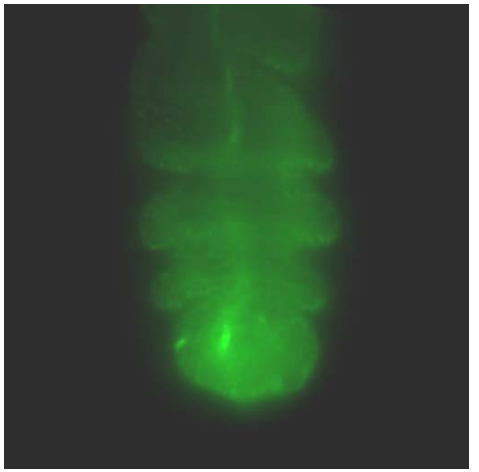


- **Filters cubes control everything**
- **One cube for one dye**
- **We need 3-7 filter cubes to get a FISH image**
- **Fluorochrome change, filter cube change**

Incident light Fluorescence







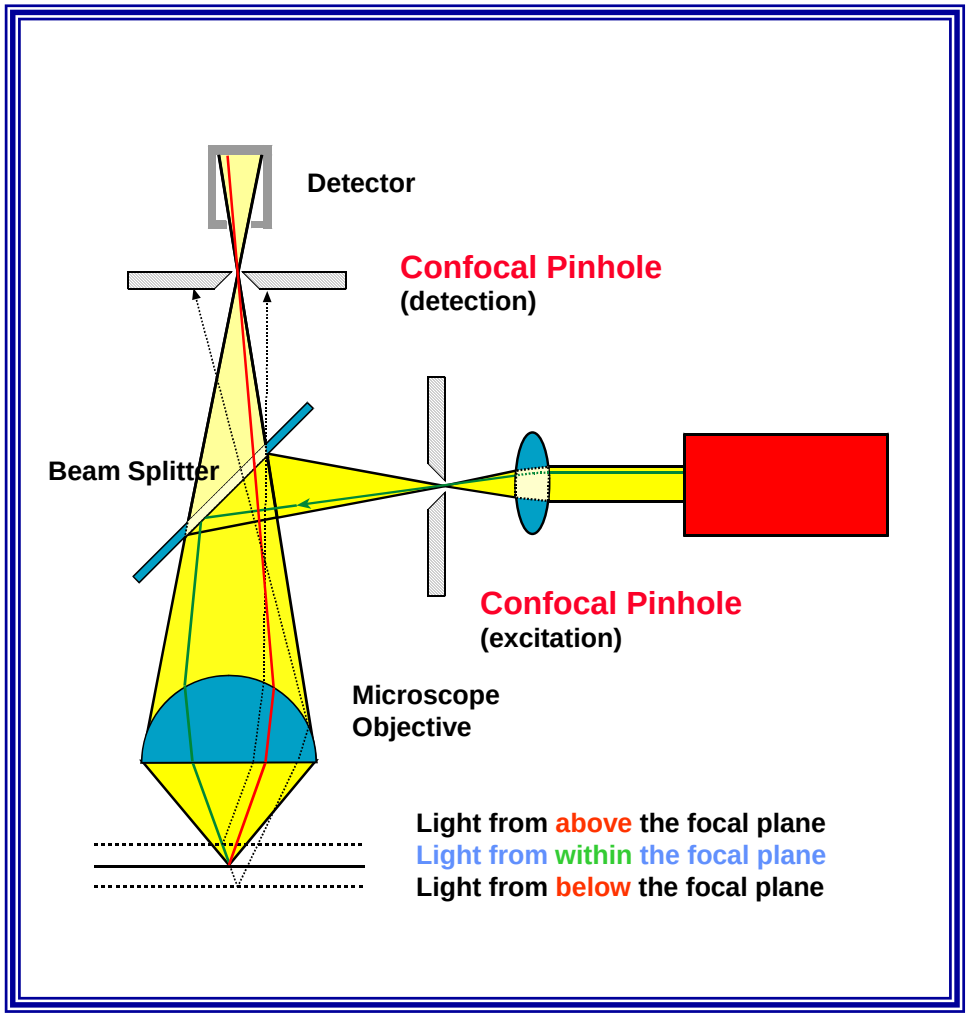
HBO 50W/100W
XBO 75W

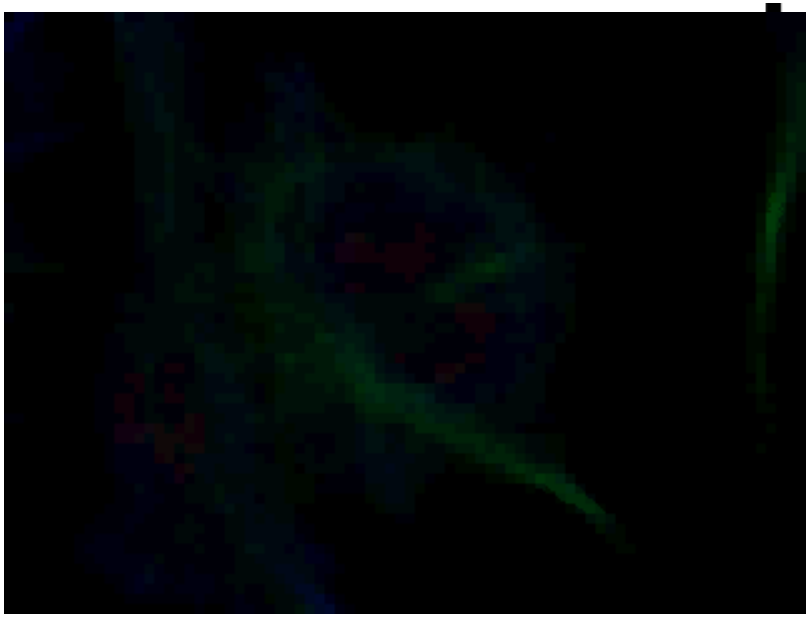
Questions:

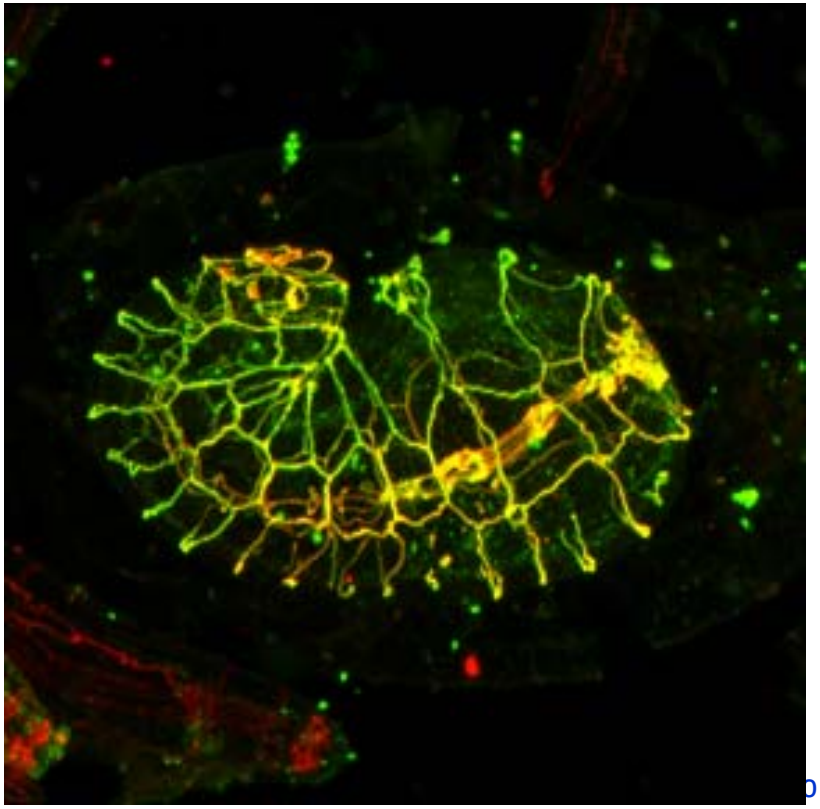
1. Image Quality?
2. Flexibility?
3. Transmission?

So, We need a " good " confocal microscope!!

LEICA Confocal Microscopy

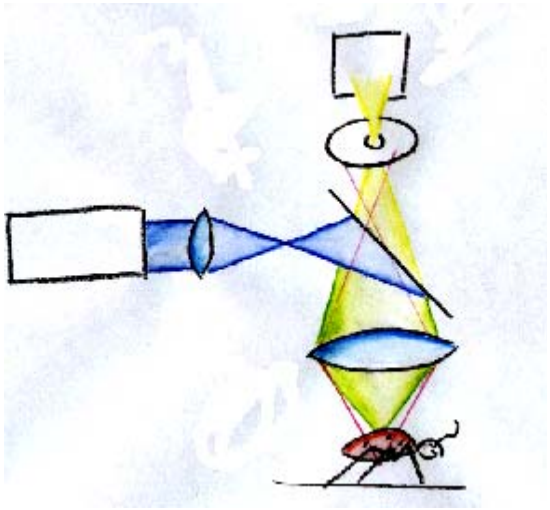






Different Techniques of Confocal Microscope

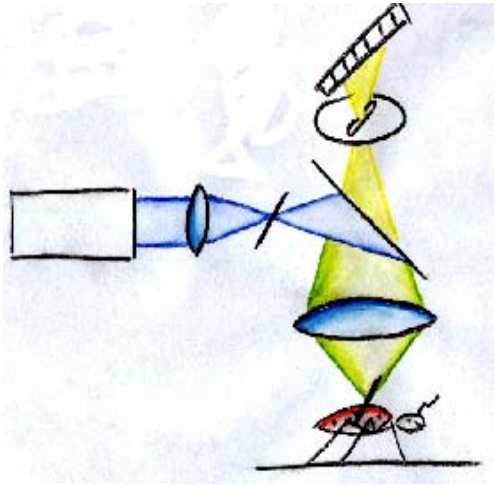
Leica
MICROSYSTEMS



point confocal

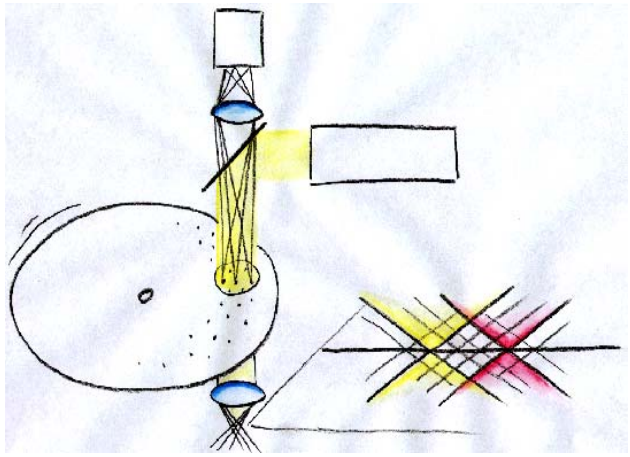
- *Point scan confocal is "true confocal"*
- *Best resolution and out of focus suppression is achieved only by classical single point confocal*

++ contrast
++ resolution
- speed



slit confocal

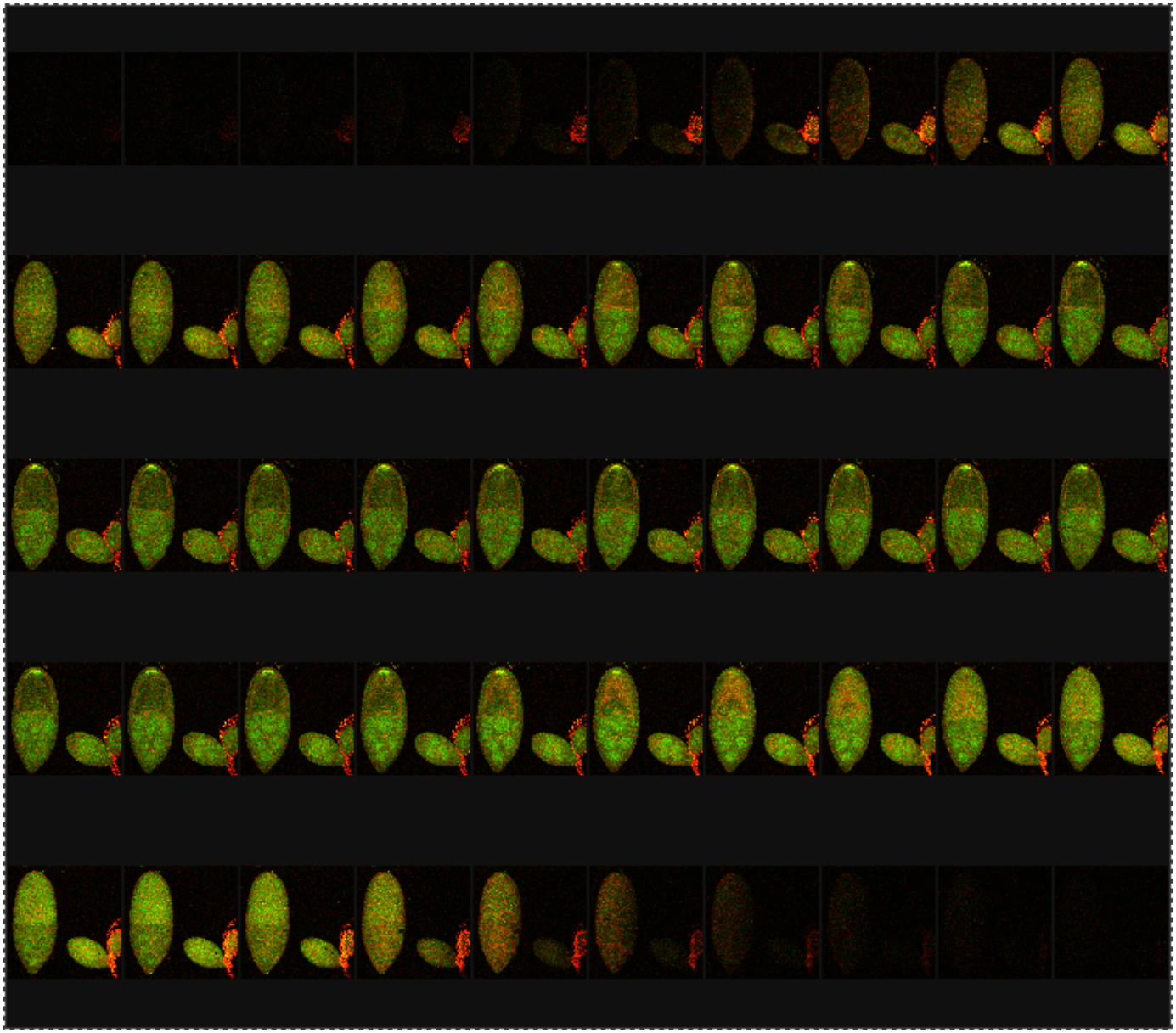
- contrast
- resolution
+ speed



Nipkov-disk scanner

- - contrast
- - resolution
++ speed

Why confocal microscopy ?

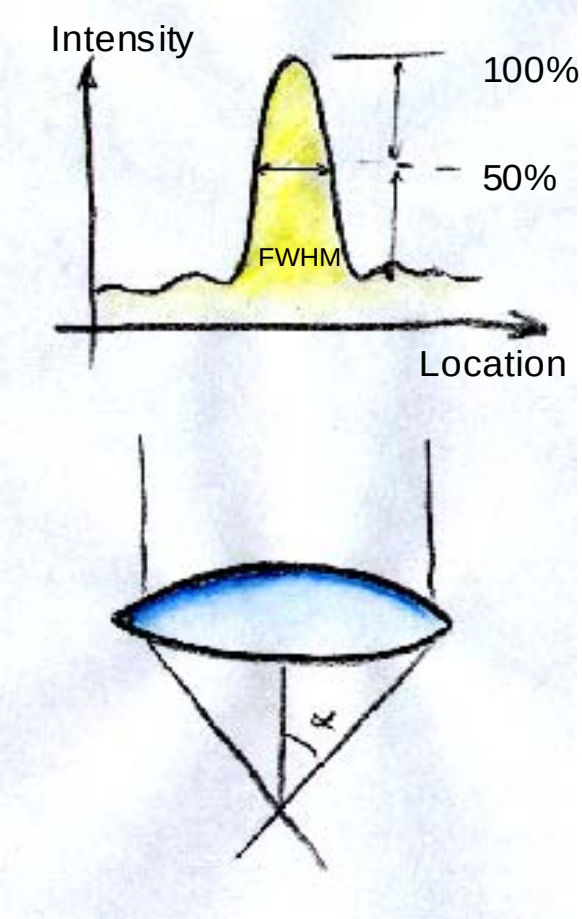


Why confocal microscopy ?

Improved resolution power (PSF) :

lateral resolution improved by approx. 1.4

Real axial resolution power



$$FWHM_{xy} = \frac{0.4\lambda}{n \cdot \sin\alpha}$$

$$FWHM_{z} = \frac{0.45\lambda}{n(1-\cos\alpha)}$$

$$NA = n \cdot \sin a$$

$n = 1$ for air

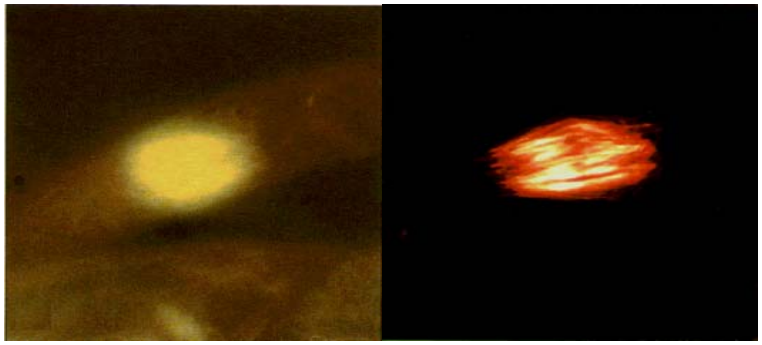
$n = 1.518$ for oil immersion

Why confocal microscopy ?

Leica
MICROSYSTEMS

Improved contrast:

Stray light due to scattering is suppressed



Multi-dimentional acquisition with digital image processing

X-Y-Z-T-I-θ-λ-n

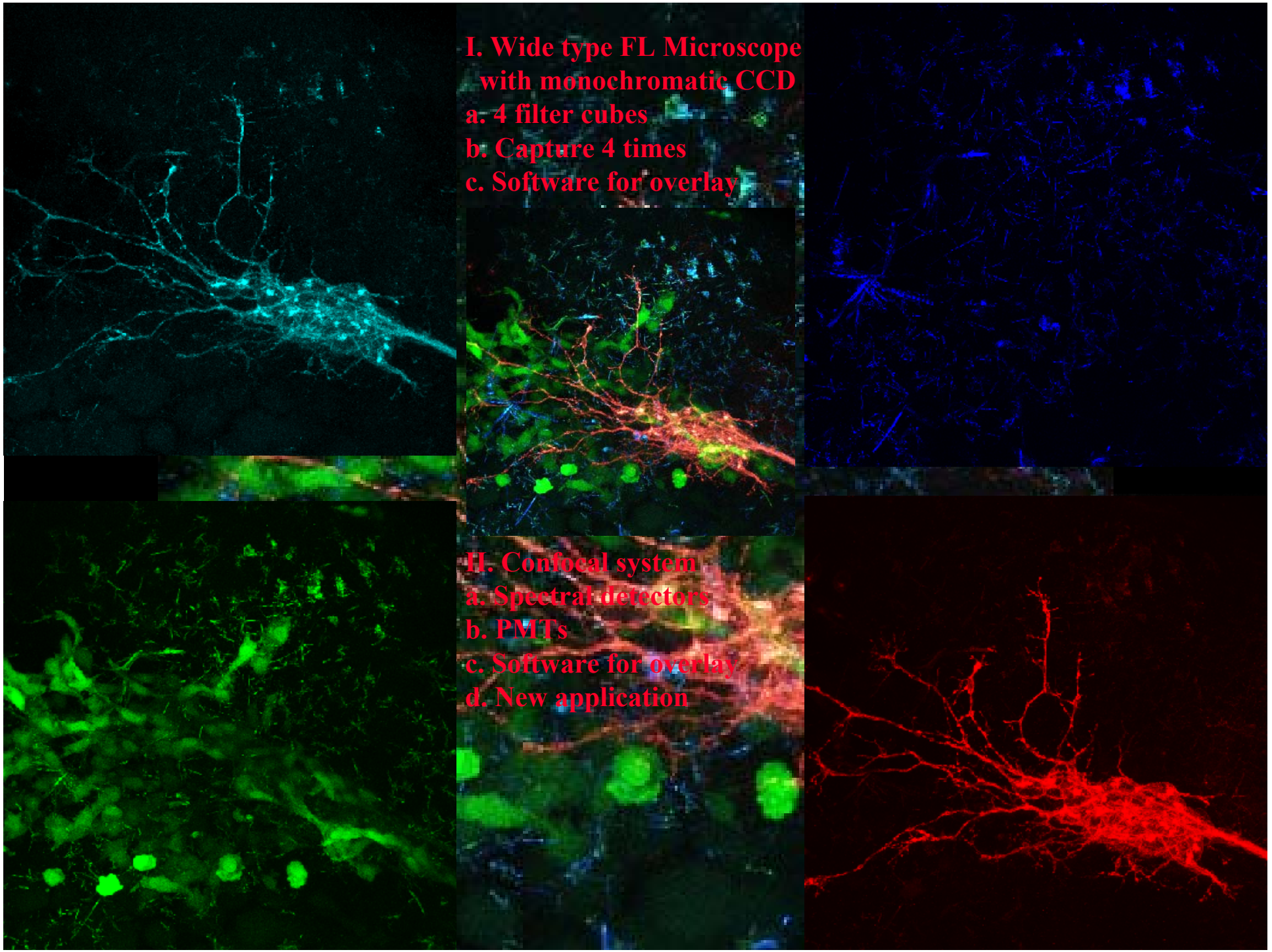
2D: xy, xz, xt

3D: xyz, xyt, xyl, xyn....

4D: xyzt, xyzn.....

5D: xyztn....

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


I. Wide type FL Microscope
with monochromatic CCD
a. 4 filter cubes
b. Capture 4 times
c. Software for overlay
II. Confocal system
a. Spectral detectors
b. PMTs
c. Software for overlay
d. New application

Leica
MICROSYSTEMS

Confocal Microscope Development

- Universal Confocal System ⇒ UV (405nm)-Vis-IR all in one
- Sensitivity and Flexibility ⇒ 1993-AOTF,
  1997-Spectral detector *(Leica patent)*
- More visible lasers merge ⇒ Whole excitation range
- Fully tunable devices ⇒ 2002-AOBS *(Leica patent)*
- Uniting two worlds- ⇒ 2005-TCS SP5 *(Leica patent)*
- Tuning into any excitation ⇒ White Light Laser System

Leica
MICROSYSTEMS

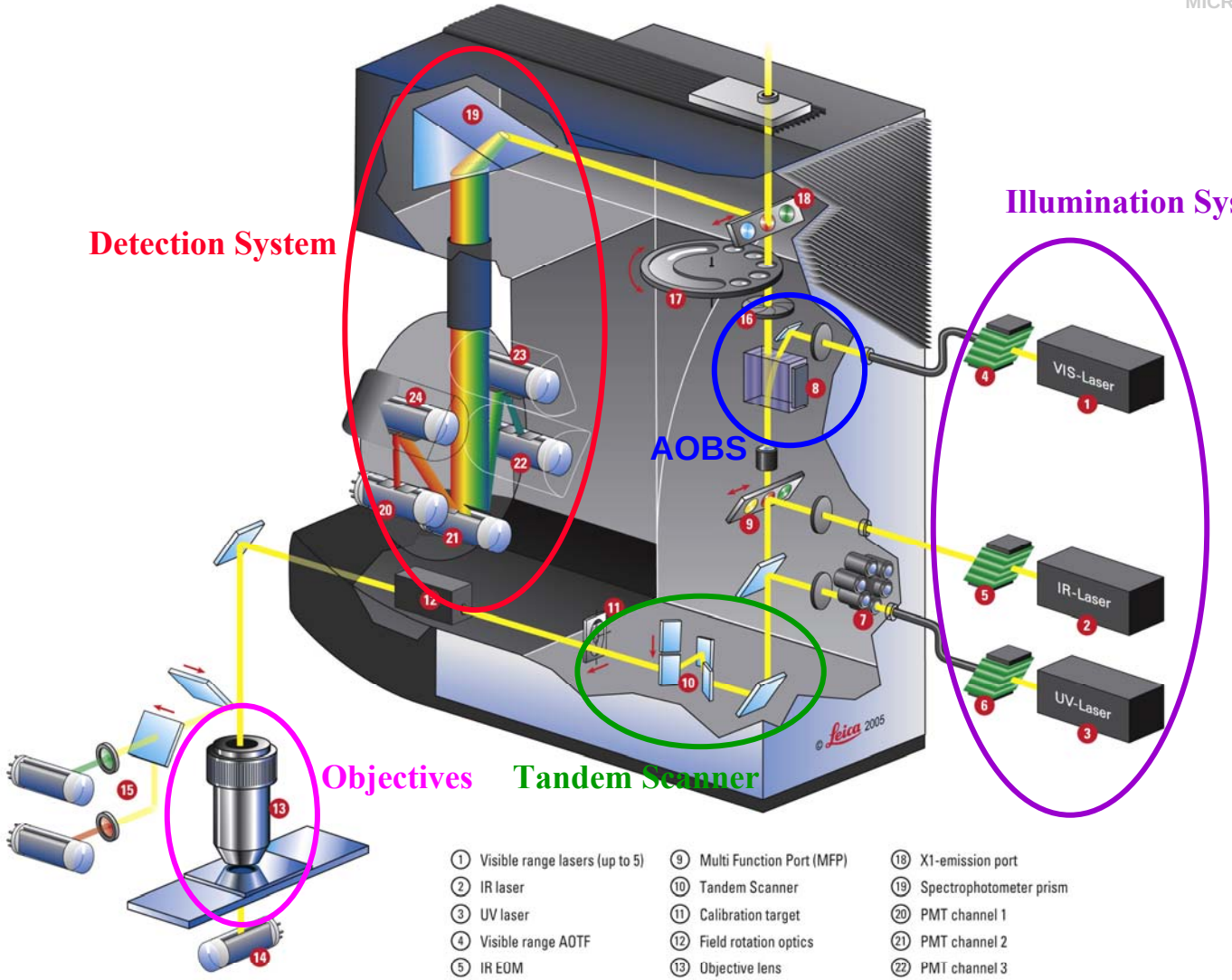
Confocal System

Main configuration :

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

LEICA Confocal Microscopy

Leica
MICROSYSTEMS



1. Visible range lasers (up to 5)
2. IR laser
3. UV laser
4. Visible range AOTF
5. IR EOM
6. UV AOTF
7. UV optics imaging
8. AOBS
   Acousto Optical Beam Splitter
9. Multi Function Port (MFP)
10. Tandem Scanner
11. Calibration target
12. Field rotation optics
13. Objective lens
14. Transmitted light dedector
15. Reflected light NDD
16. Confocal detection pinhole
17. Filter- and polarizer wheel
18. X1-emission port
19. Spectrophotometer prism
20. PMT channel 1
21. PMT channel 2
22. PMT channel 3
23. PMT channel 4 (optional FLIM)
24. PMT channel 5 (optional FLIM)

Leica
MICROSYSTEMS

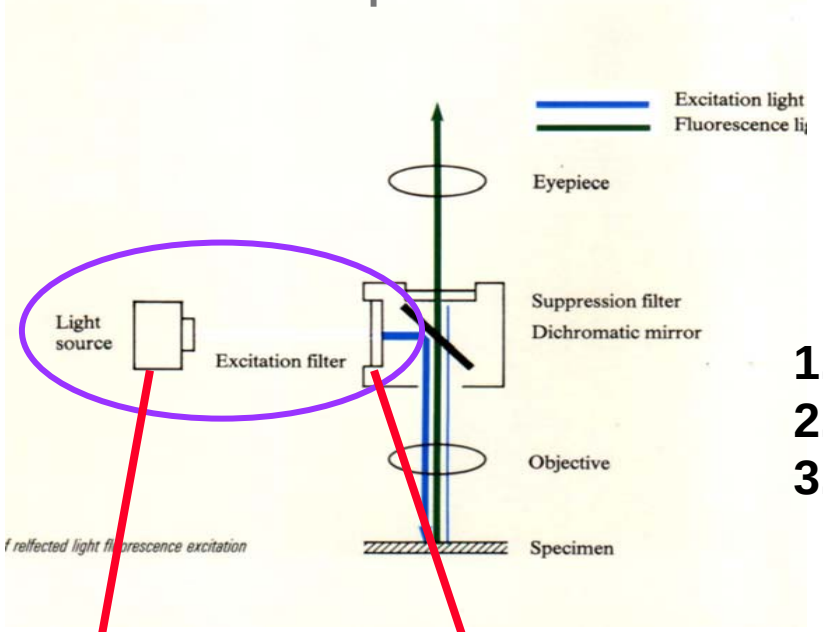
Illumination (Excitation) System

- Filter base system

- AOTF system

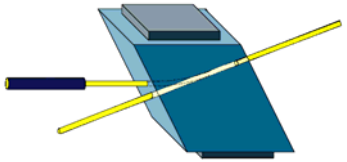
Ilumination System

Conventional Fluorescence Microscope vs. Confocal Microscope



1. **Intensity Control?**
2. **Multiline?**
3. **Flexibility?**

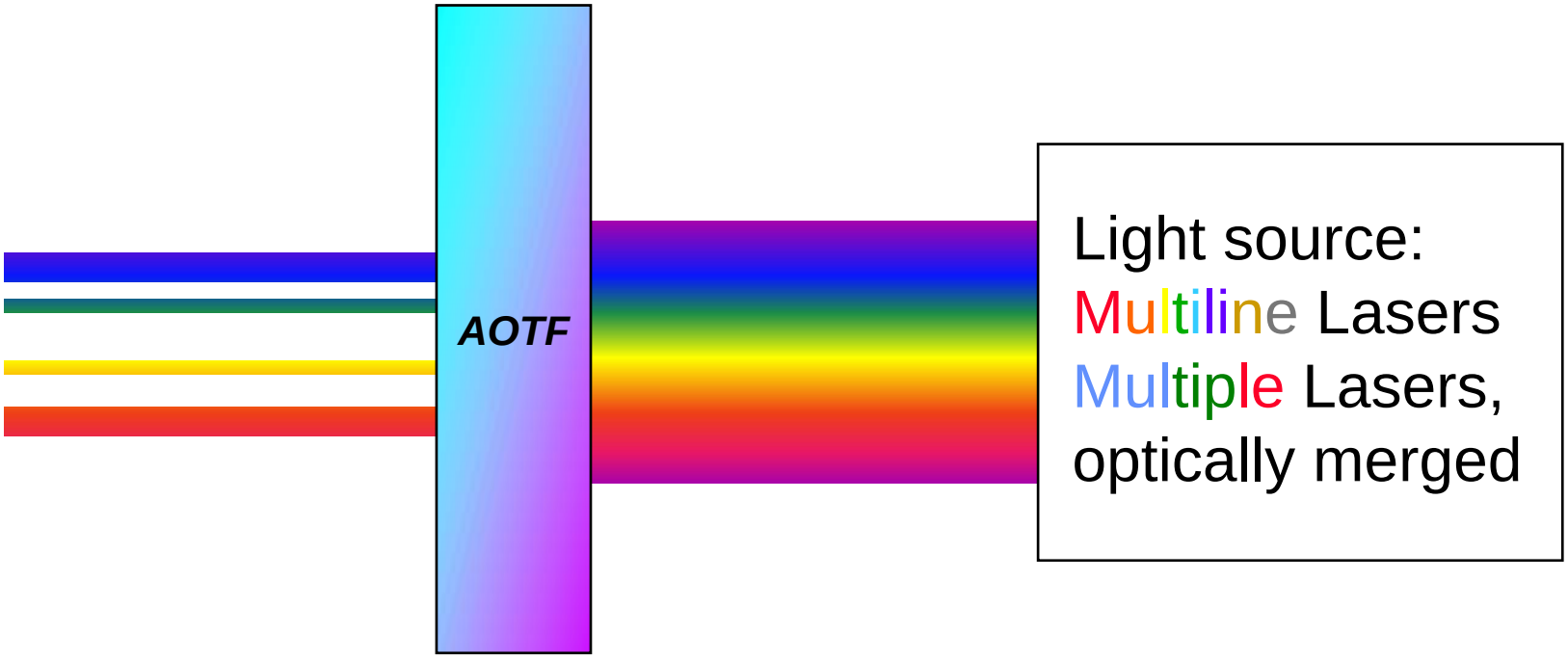
Multiline Lasers
Multiple Lasers



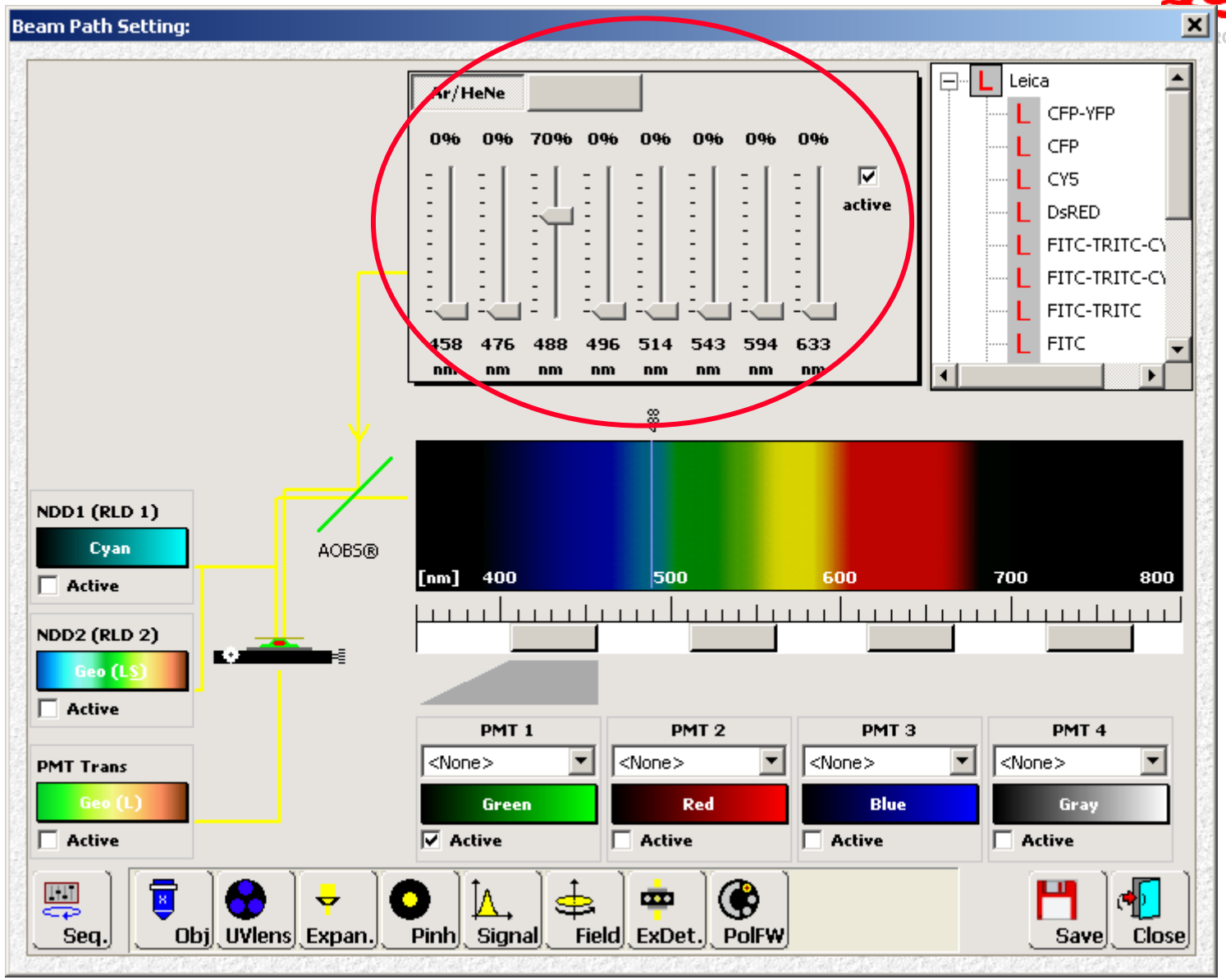
Leica 1993
Excitation control: AOTF

AOTF - Variable Excitation Filter

Acousto Optical Tunable Filter

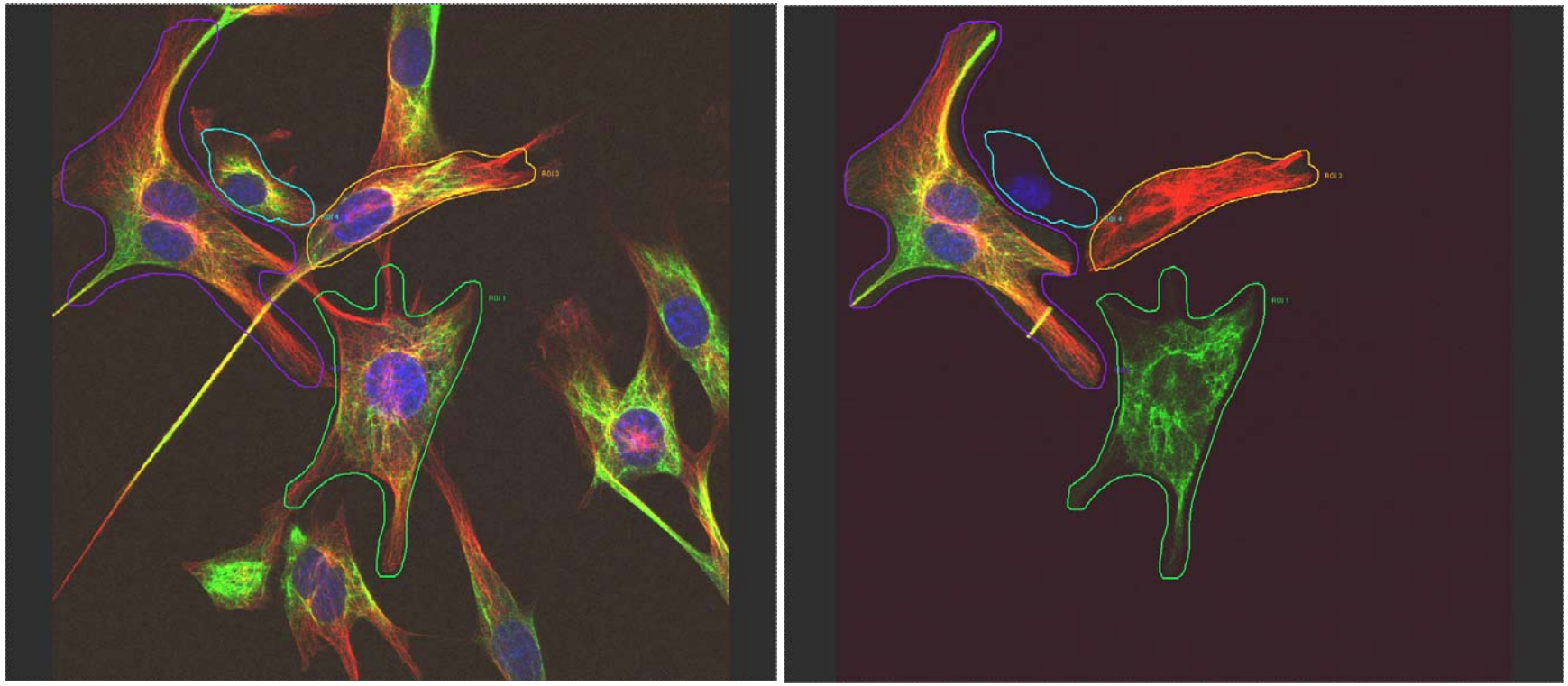


- ✓ Independent line selection
- ✓ Independent intensity control


Beam Path Setting:
Ar/HeNe
0% 0% 70% 0% 0% 0% 0% 0%
active
458 476 488 496 514 543 594 633
nm nm nm nm nm nm nm nm
Leica
CFP-YFP
CFP
CY5
DsRED
FITC-TRITC-CY
FITC-TRITC-CY
FITC-TRITC
FITC
AOBS®
[nm] 400 500 600 700 800
NDD1 (RLD 1)
Cyan
Active
NDD2 (RLD 2)
Geo (LS)
Active
PMT Trans
Geo (L)
Active
PMT 1
<None>
Green
Active
PMT 2
<None>
Red
Active
PMT 3
<None>
Blue
Active
PMT 4
<None>
Gray
Active
Seq.
Obj
UVlens
Expan.
Pinh
Signal
Field
ExDet.
PolFW
Save
Close

Leica MICROSYSTEMS

ROI (region of interest)



- Freely configurable laser lines and intensities for ROI´s and surrounding area
- FRAP
- Uncaging

Fibroblasts
ROI 1 543 Cy3 (Intermediate Filaments)
ROI 2 all lines
ROI 3 488 FITC (Microtubules)
ROI 4 UV DAPI (Nucleus)

LEICA Confocal Microscopy

Leica MICROSYSTEMS

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…
➲ 2005 HP Blue	Argon, 300mW	458, 476, 488, 496*, 514 nm	
√ Green	HeNe, 1mW	543 nm	Alexa 546, 555, 568, Cy3, TRITC, DsRed..
➲ 2004 Light Green	DPSS, 20mW	561 nm	
	Kr, 25mW	568 nm	
➲ 2003 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

*** Only for LEICA AOBS System !!**

Leica MICROSYSTEMS

UV – 405 nm Lasers

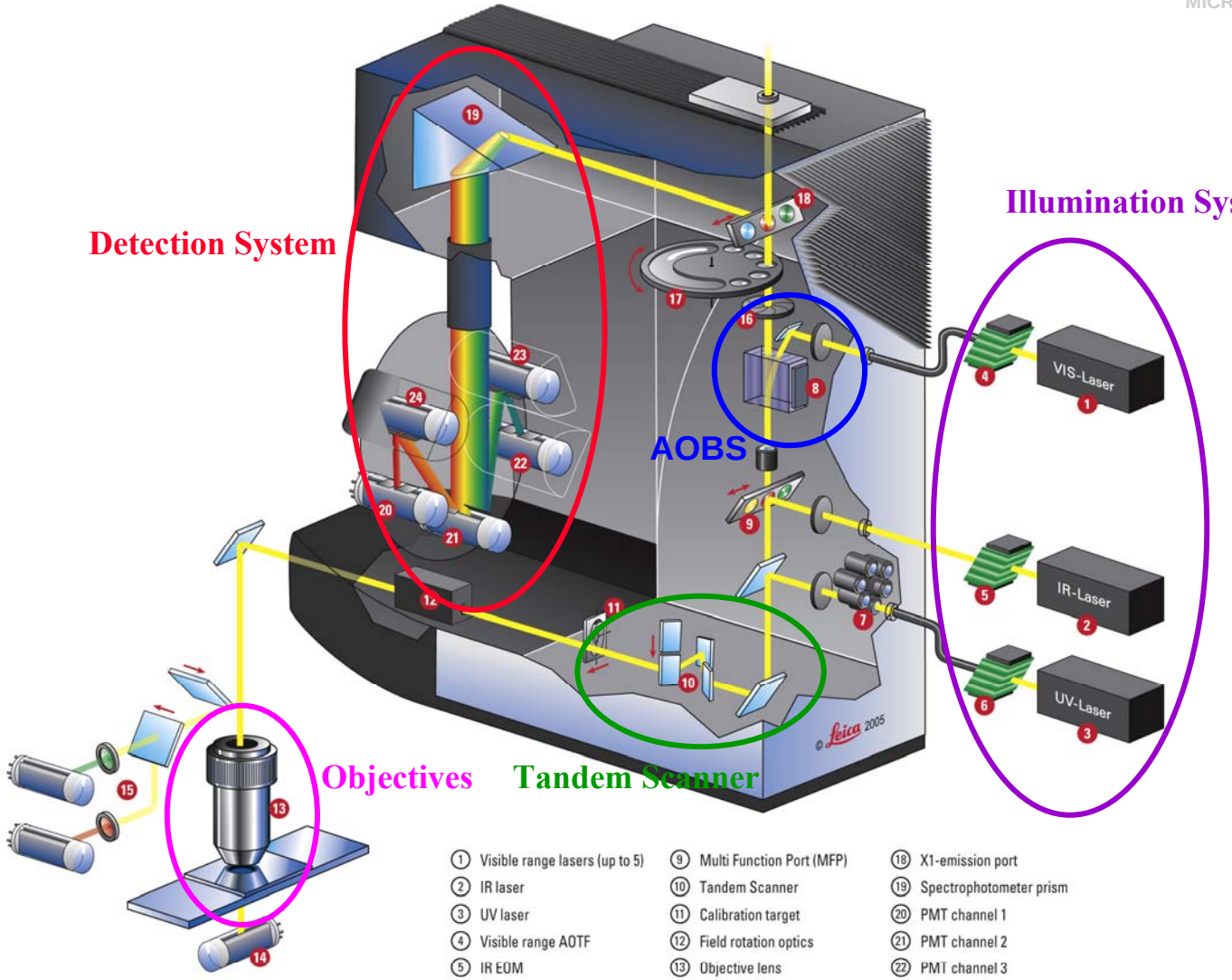
	Type	Wavelength	Fluorochrome
UV	Argon, 50mW	351, 364 nm	DAPI, Hoechst...
2003 ➲ Violet	Diode, 25mW	405 nm	DAPI, Hoechst, BFP, CFP...
2005 ➲ HP Violet	Diode, 50mW	405 nm	

Diode Laser 405 nm

- Need not extra cooling system, cheaper than Ar-UV laser.
- Higher lifetime than Ar-UV laser.
- Not only for DAPI and Hoechst®, but also covers CFP and GFP2 (BFP), components of the important FRET pairs CFP/YFP and GFP2/YFP.
- UV pinhole lens needed.

LEICA Confocal Microscopy

Leica
MICROSYSTEMS



1. Visible range lasers (up to 5)
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17. Filter- and polarizer wheel
18. X1-emission port
19. Spectrophotometer prism
20. PMT channel 1
21. PMT channel 2
22. PMT channel 3
23. PMT channel 4 (optional FLIM)
24. PMT channel 5 (optional FLIM)

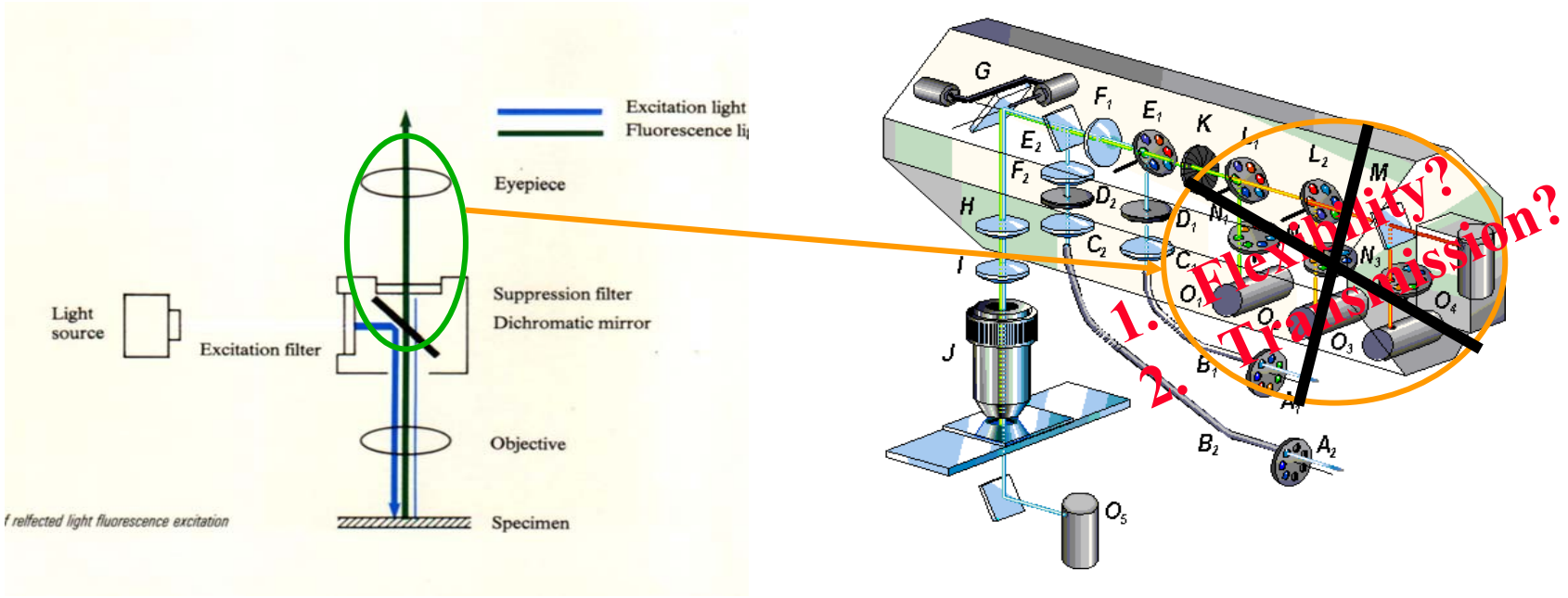
Leica
MICROSYSTEMS

Detection (Emmision) System

- Filter base detector

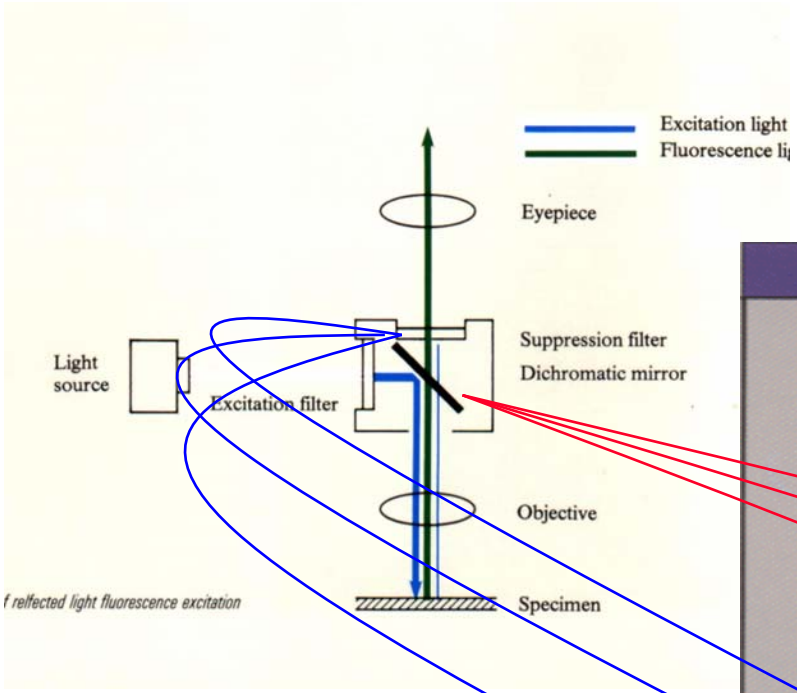
- Spectral detector

Detection System - Filter Base -



- 每一個螢光偵測器前都有一組Dichromatic mirror及Suppression filter
- 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

Filter Based Detector



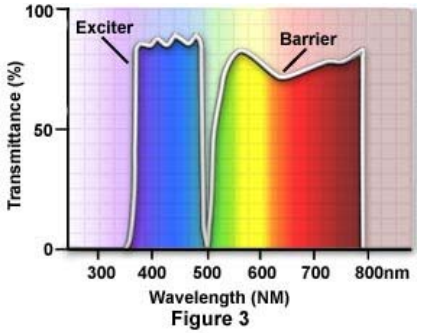
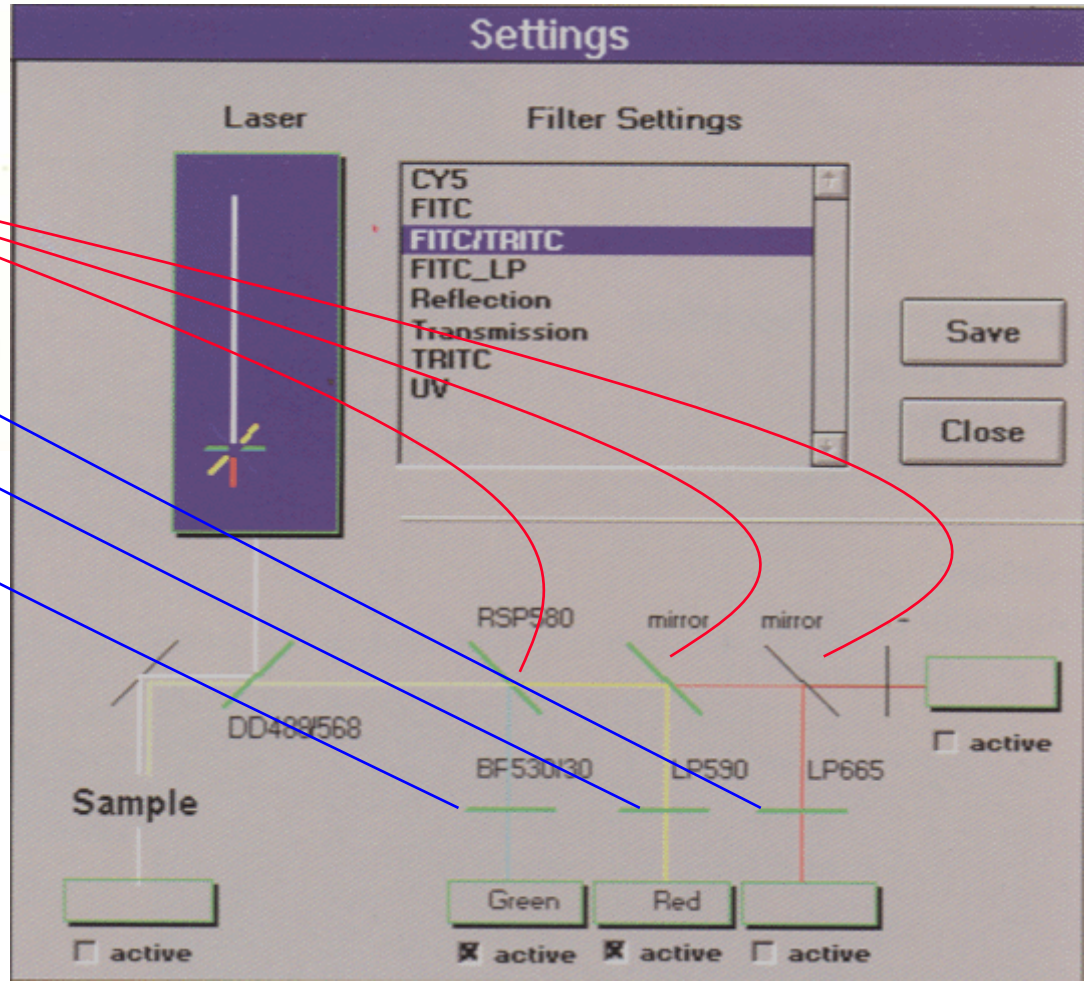


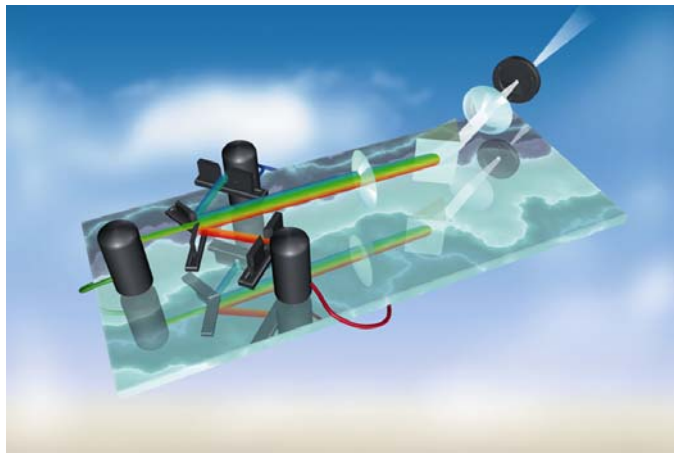
Figure 3



Detection System – Spectrum Base -

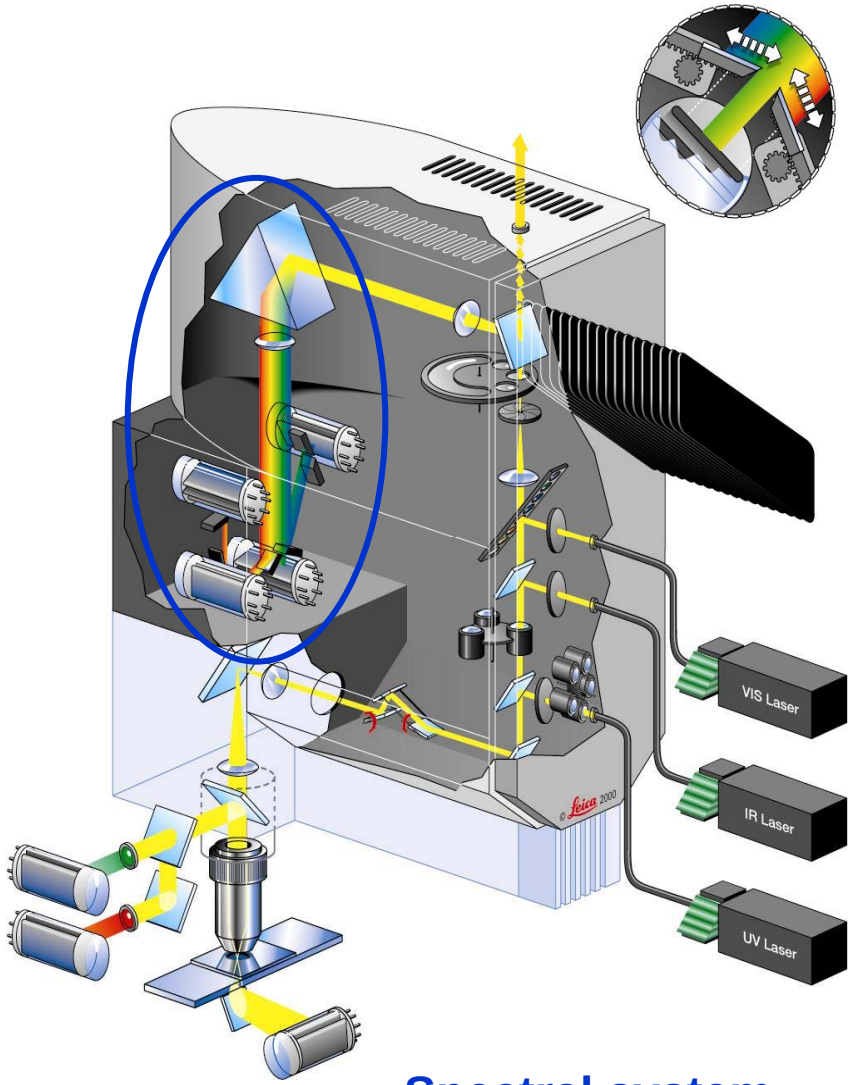
MICROSYSTEMS

Spectrum Idea:

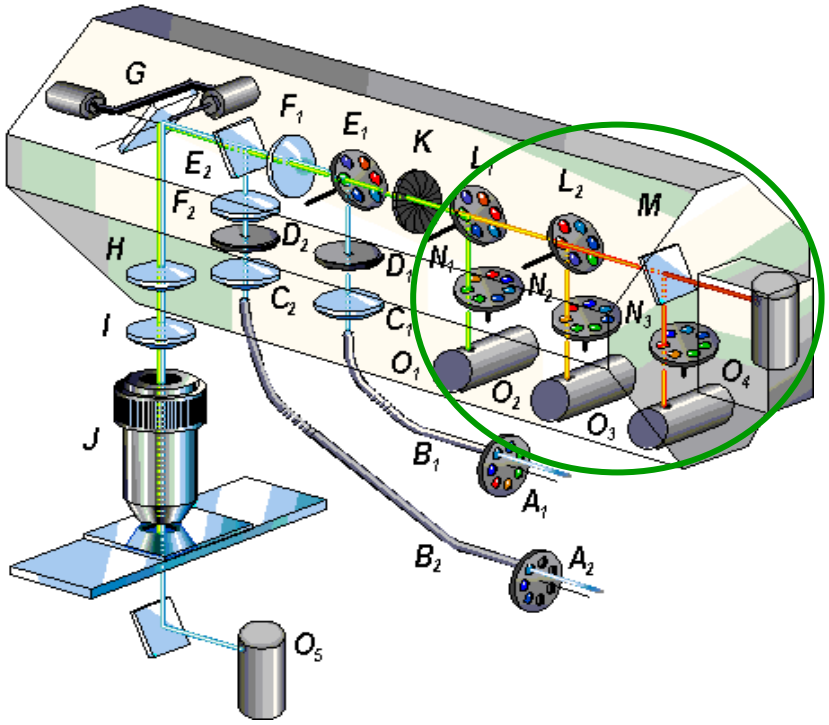


Leica 1997
Emission control:
SP Detector

- 螢光偵測器前已無任何的Dichromatic mirror及Suppression filter
- 使用光柵篩選所欲得到的螢光波長範圍
- 真正的 “Design your own filters ” - LEICA專利設計

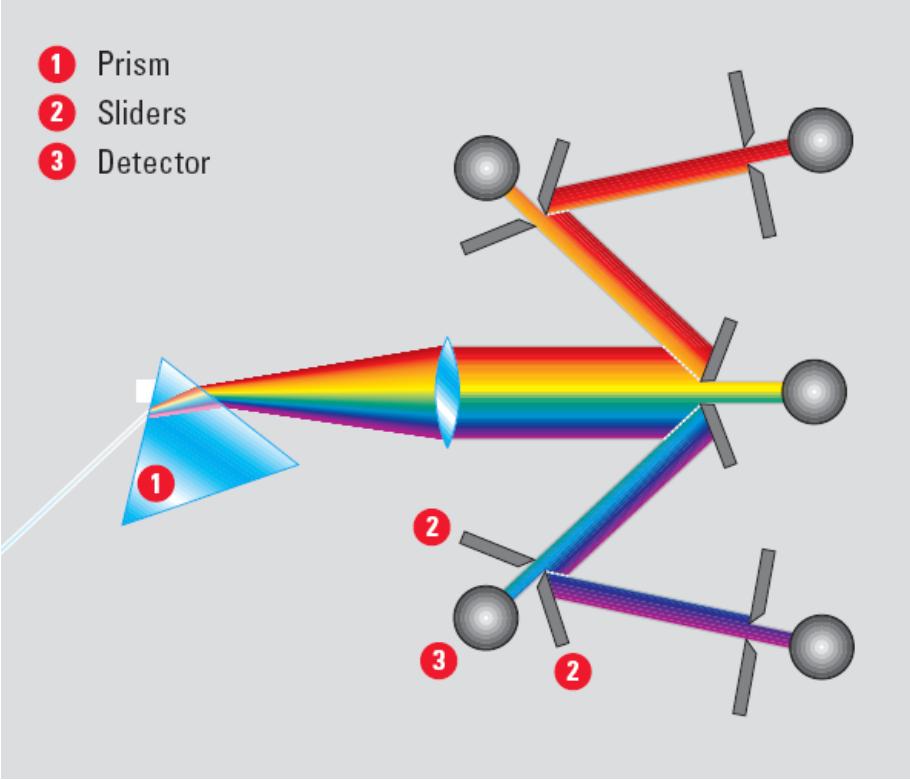


Spectral system

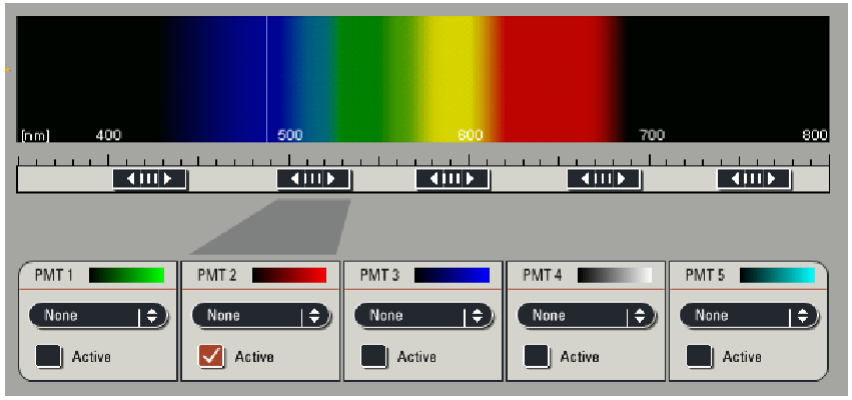


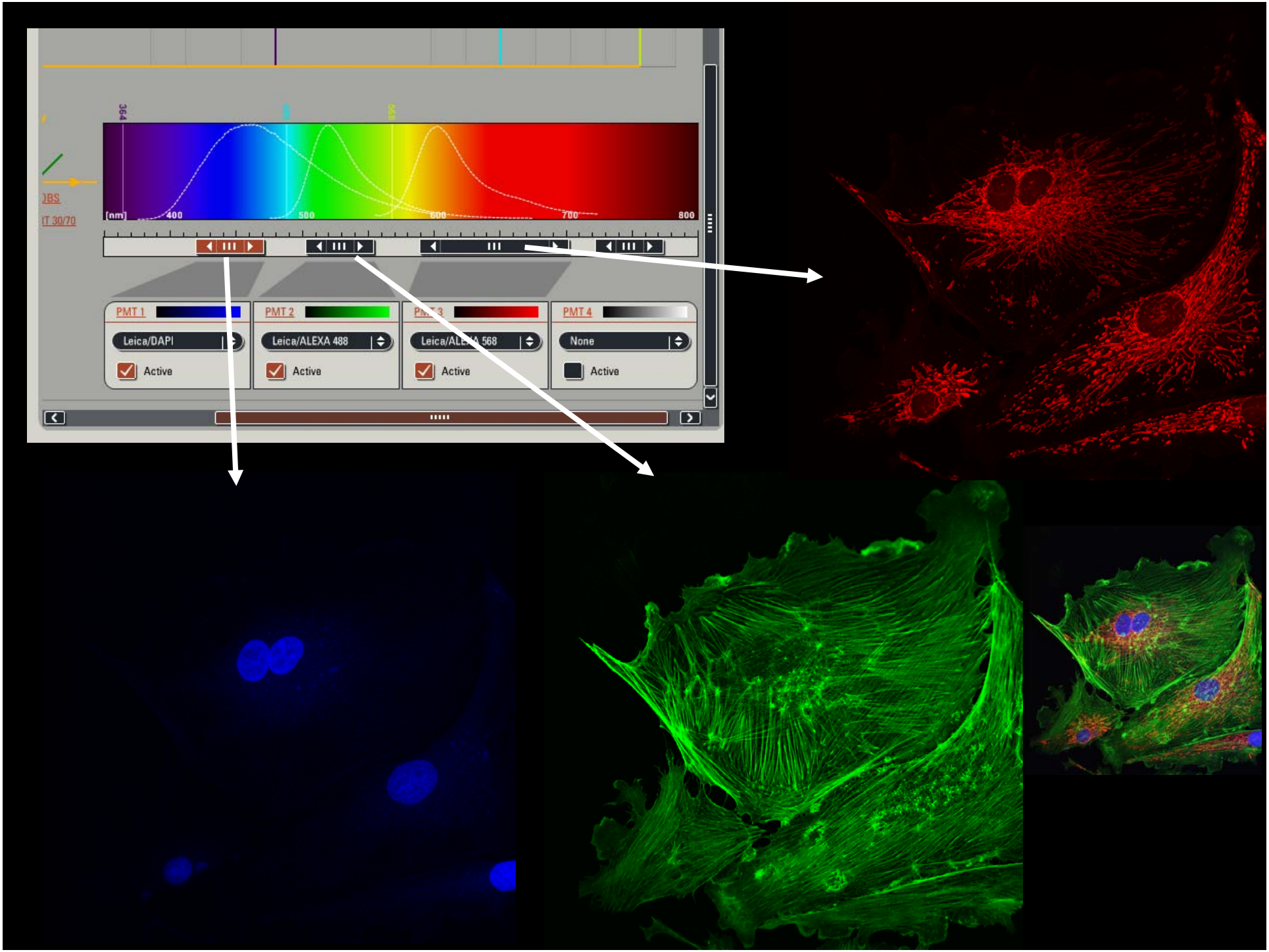
Filter base system

Leica TCS SP5 - *Leading in Multispectral Imaging:* Spectral Imaging Detector SP – *principle and benefits*



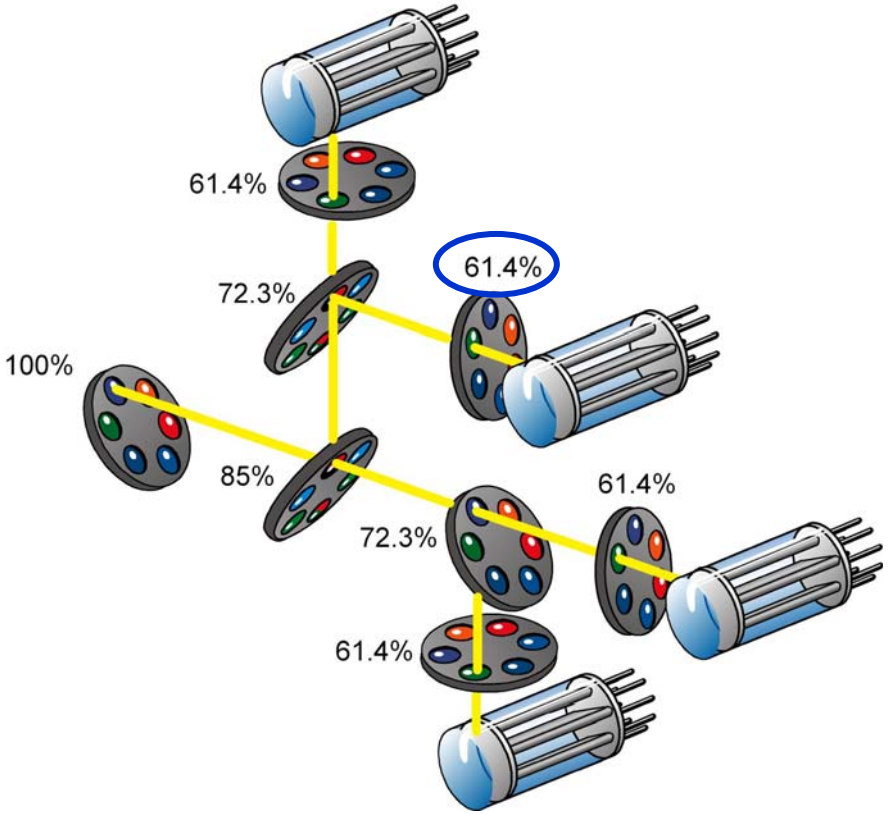
- All dyes: Freely tuneable emission bands
- Low sample photodamage: high efficiency
- 5 true confocal channels simultaneously
- Intuitive operation

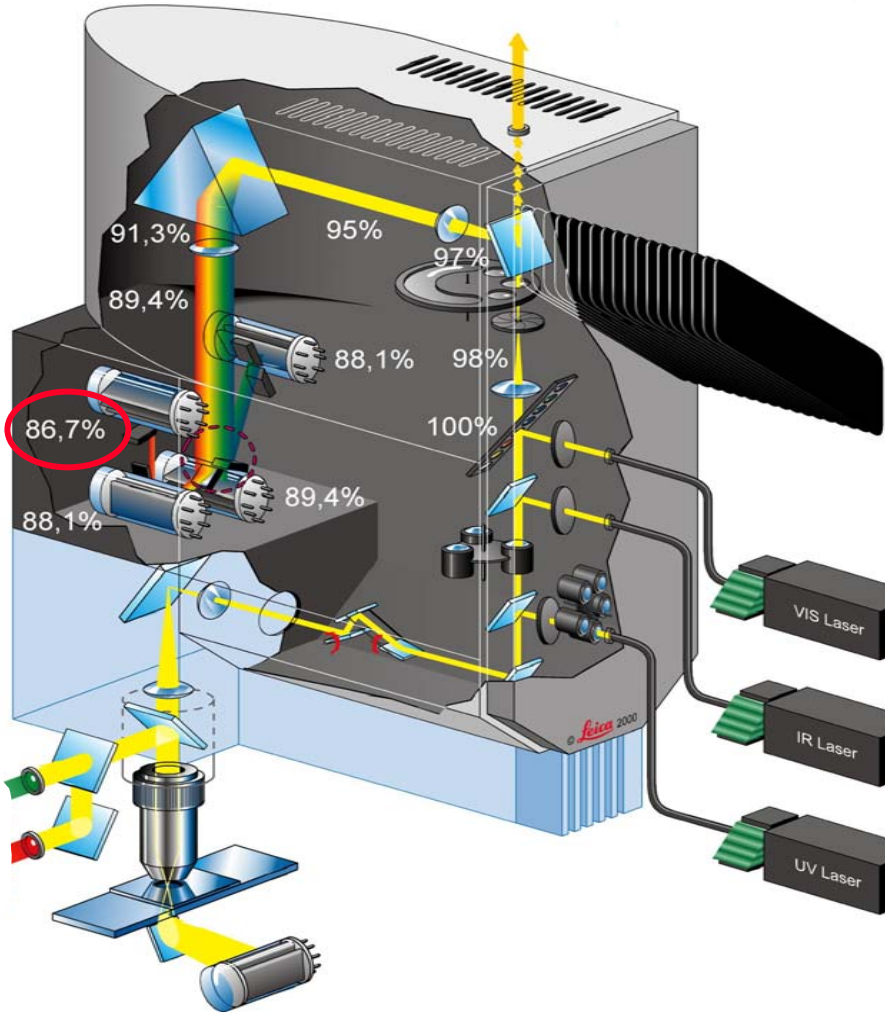



364
[nm]
400
500
600
700
800
PMT 1
Leica/DAPI
Active
PMT 2
Leica/ALEXA 488
Active
Active
PMT 4
None
Active

Filter Base vs. Spectral Base

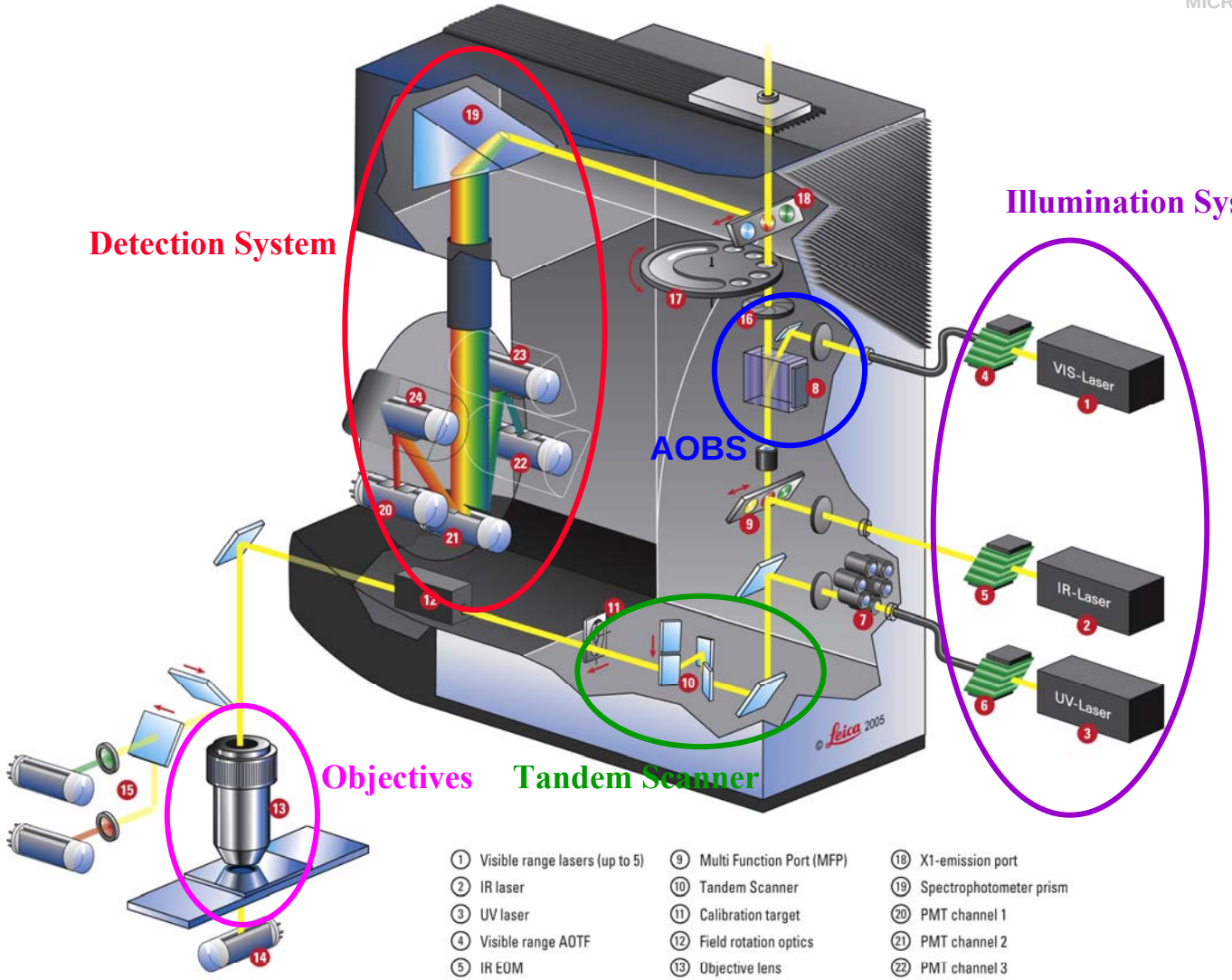
- Transmission comparison -





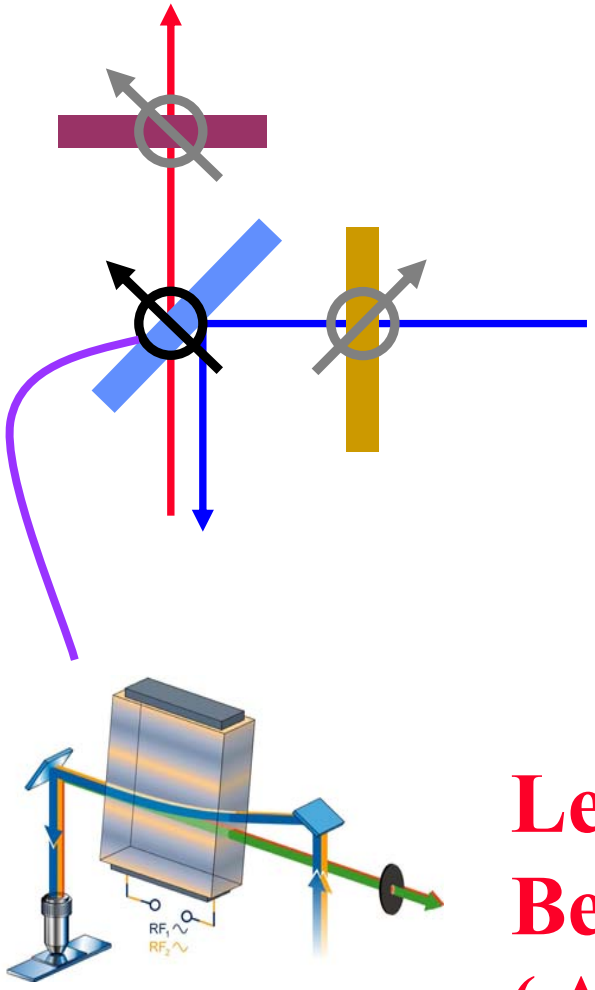
LEICA Confocal Microscopy

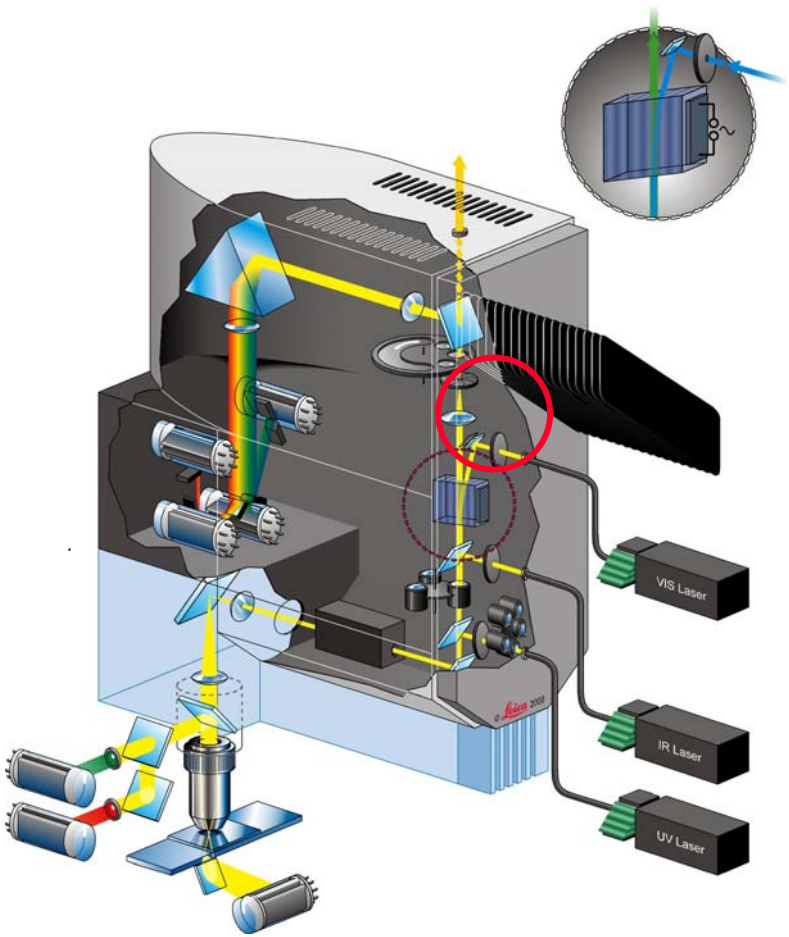
Leica
MICROSYSTEMS



1. Visible range lasers (up to 5)
2. IR laser
3. UV laser
4. Visible range AOTF
5. IR EOM
6. UV AOTF
7. UV optics imaging
8. AOBS
   Acousto Optical Beam Splitter
9. Multi Function Port (MFP)
10. Tandem Scanner
11. Calibration target
12. Field rotation optics
13. Objective lens
14. Transmitted light dedector
15. Reflected light NDD
16. Confocal detection pinhole
17. Filter- and polarizer wheel
18. X1-emission port
19. Spectrophotometer prism
20. PMT channel 1
21. PMT channel 2
22. PMT channel 3
23. PMT channel 4 (optional FLIM)
24. PMT channel 5 (optional FLIM)

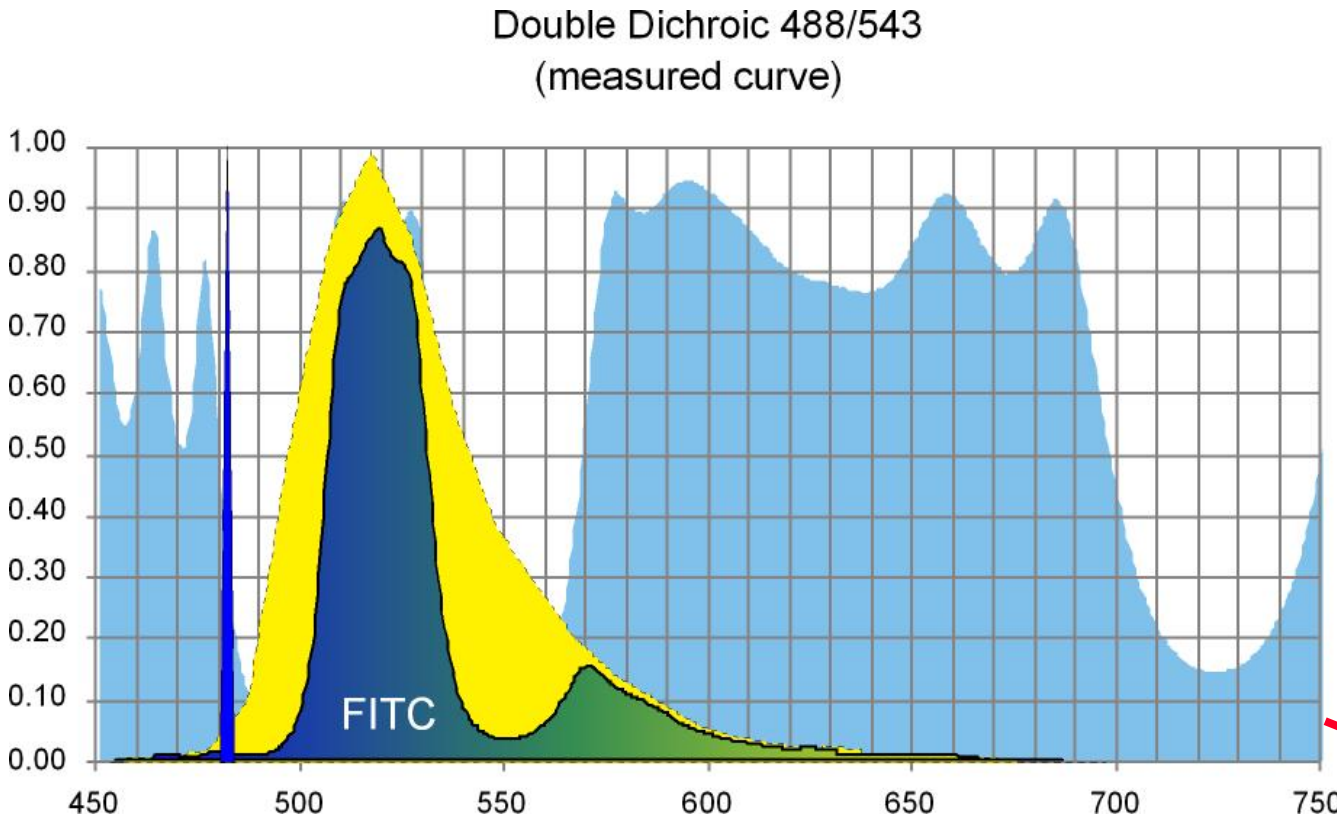
So far



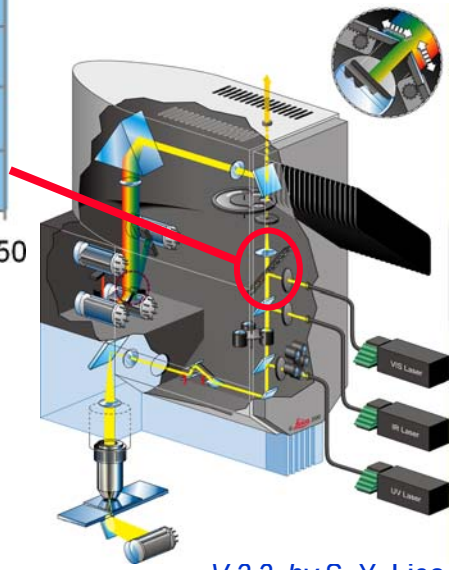


Leica 2002
Beam Splitting Control: AOBS
(Acousto Optical Beam Splitter)

Beam splitter limitations

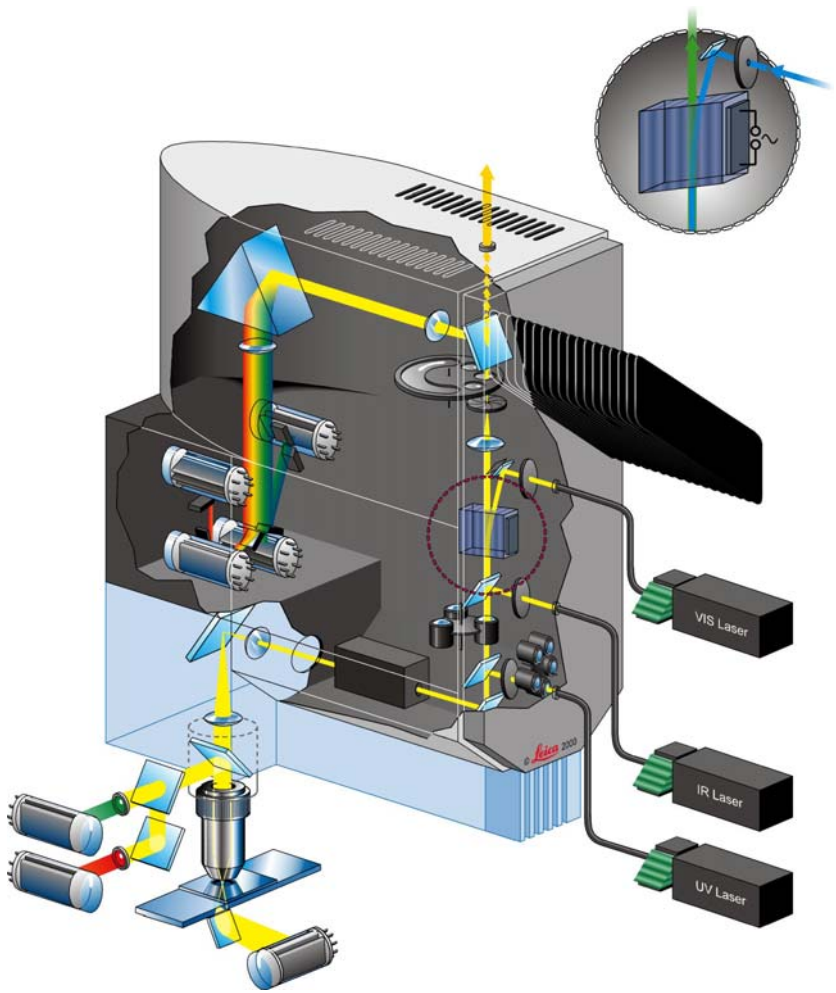


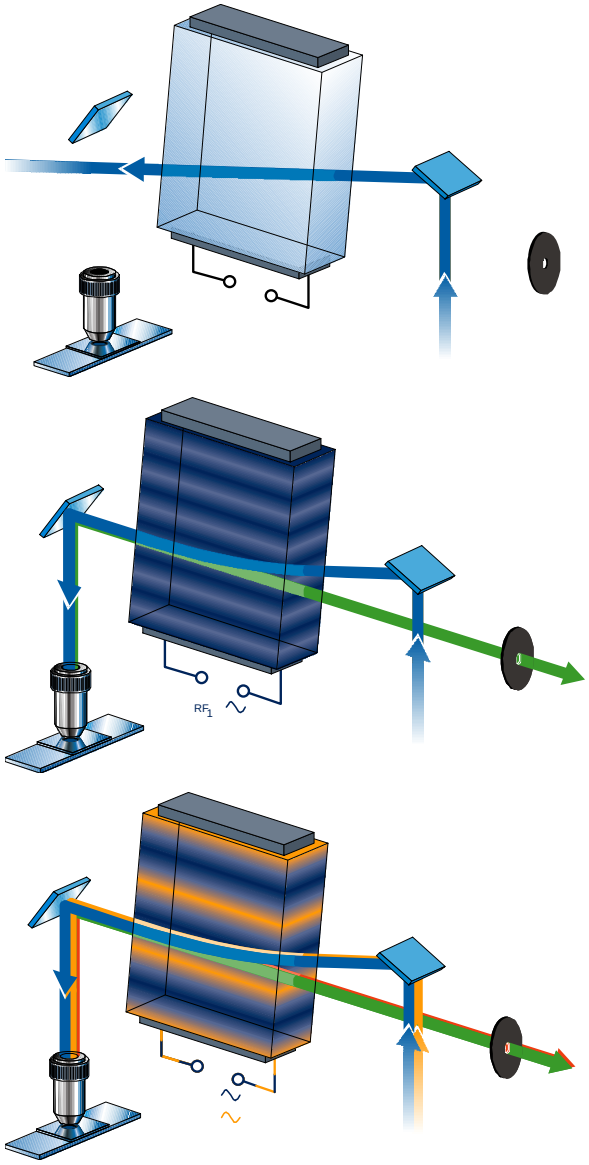
Transmission



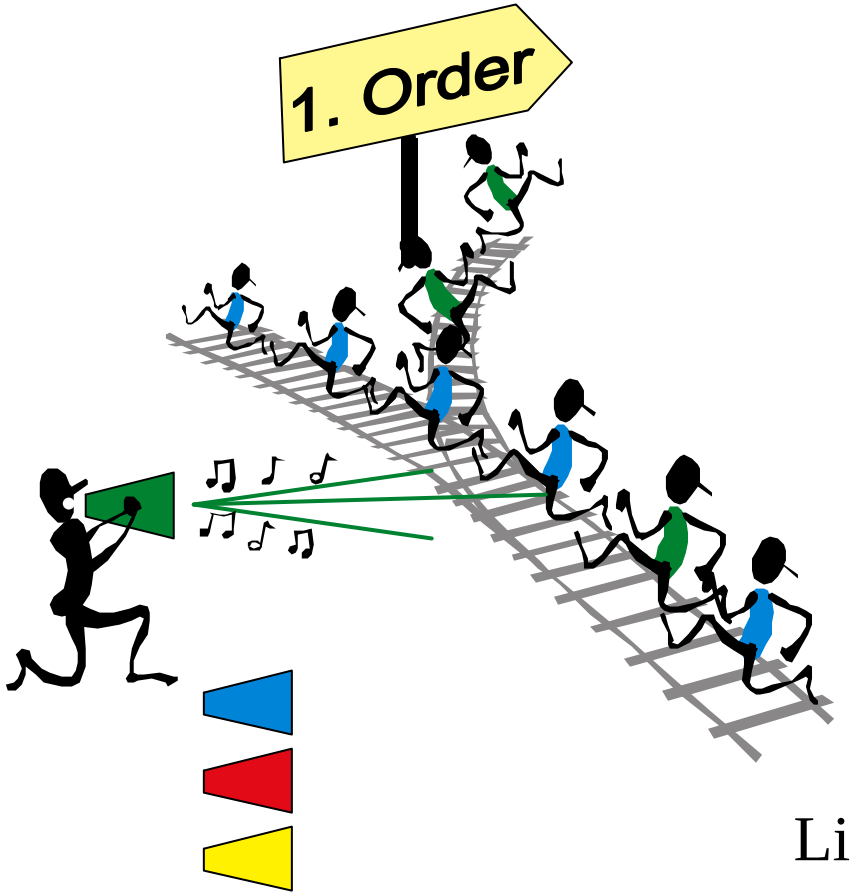
- No sharp bands
- Transmission holes
- Fixed characteristics

AOBS properties





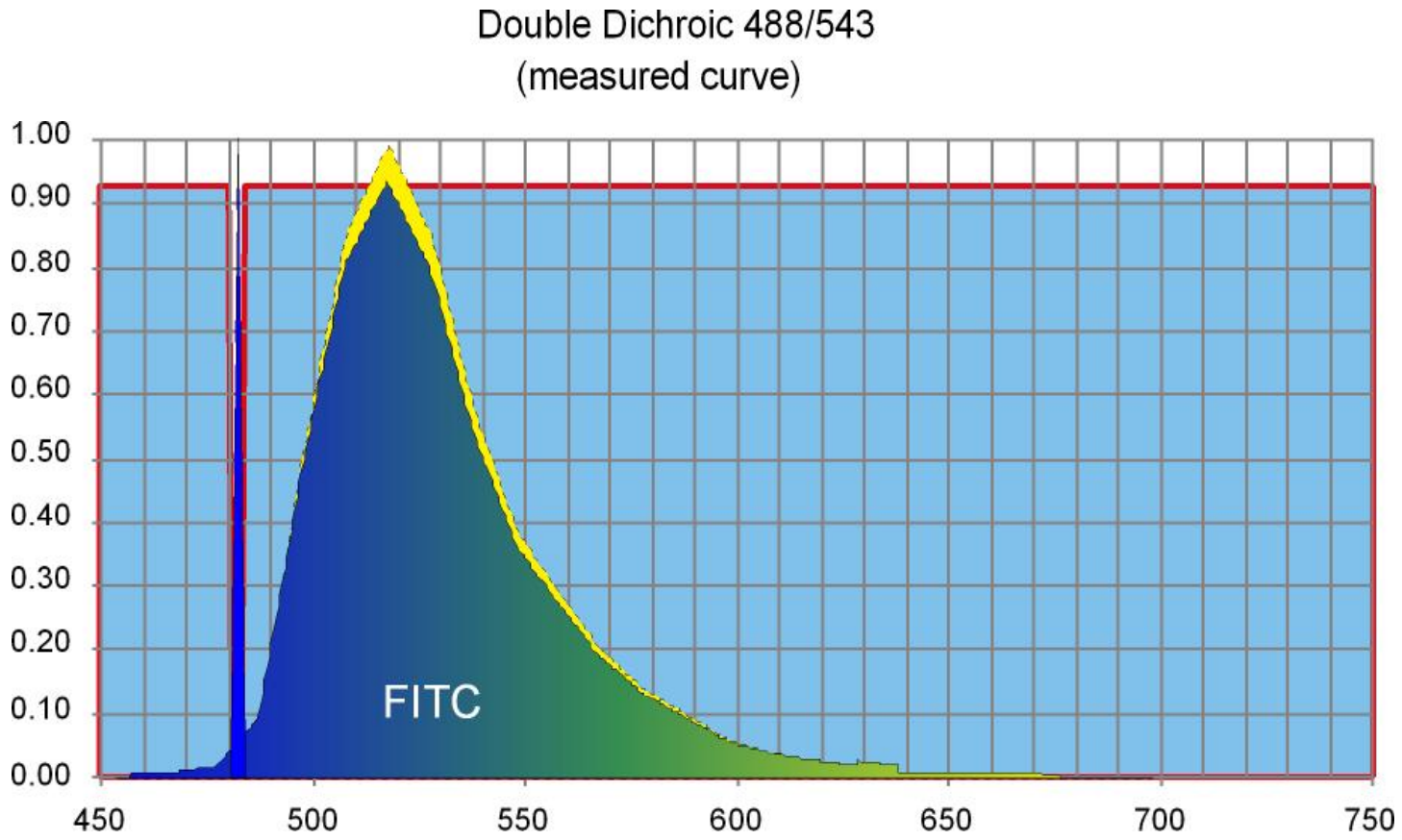
AOTF/AOBS properties



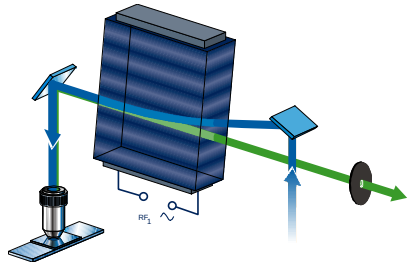
Light from Laser

Ultra-sonic wave

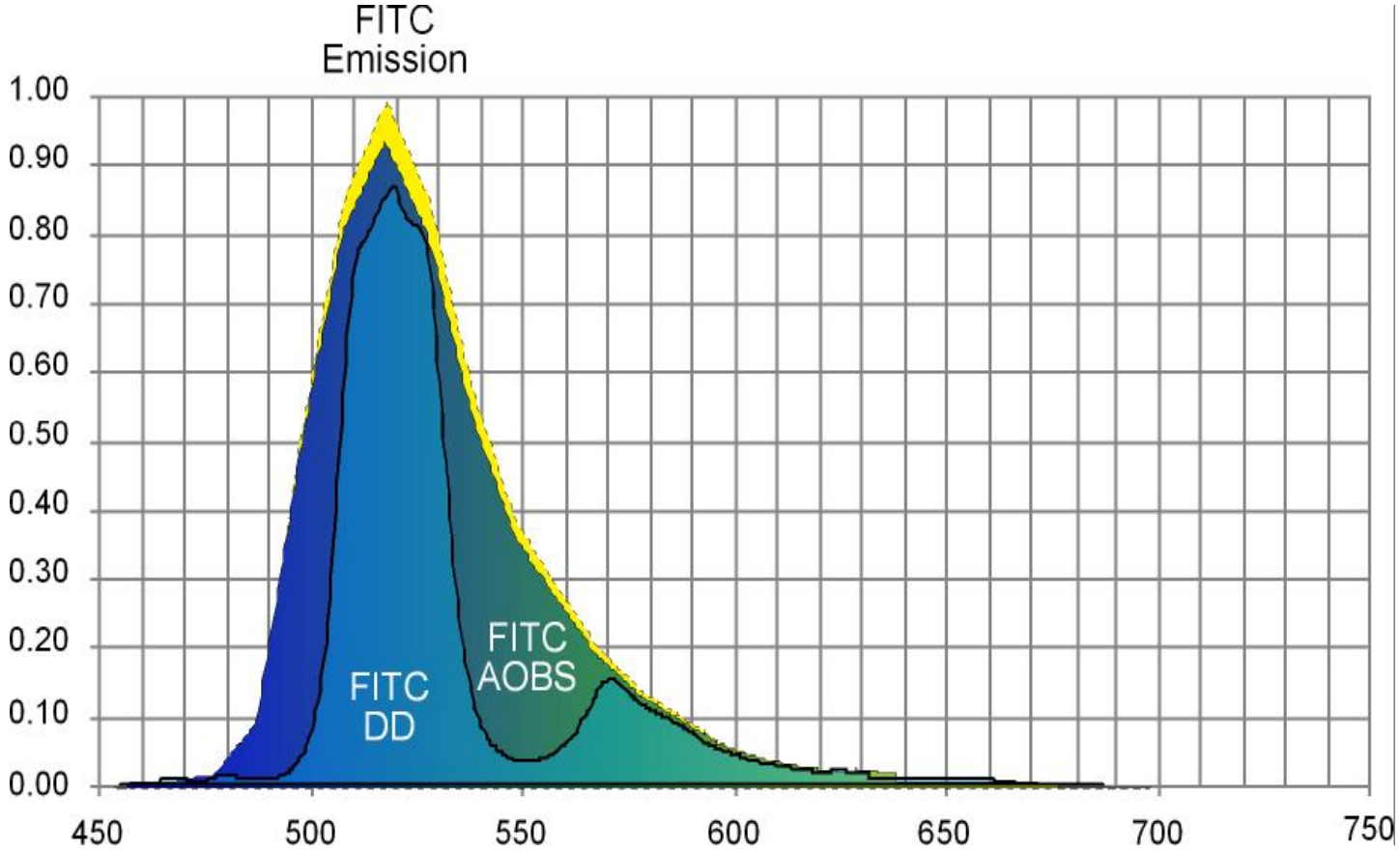
AOBS: More efficient



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

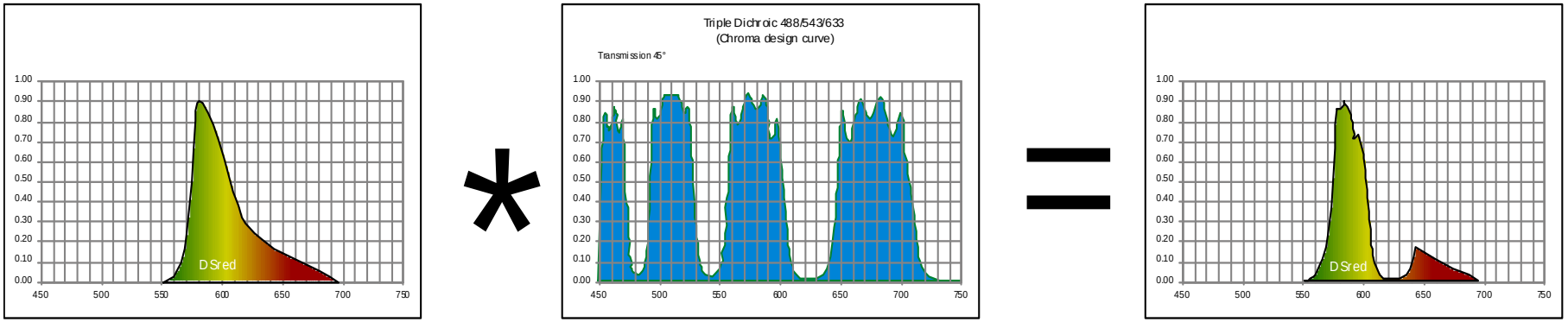


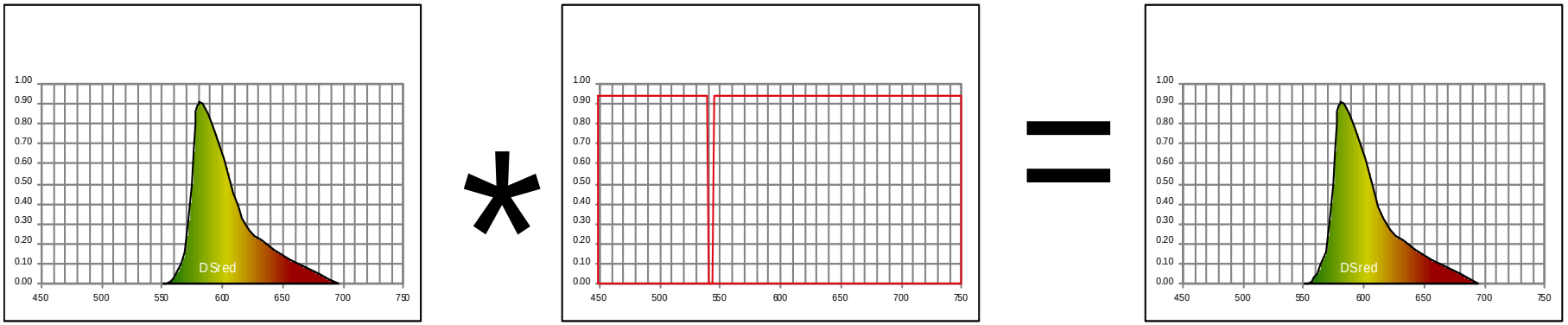
AOBS: More efficient - FITC



✓ AOBS transmits 30% more emission light than beam splitter

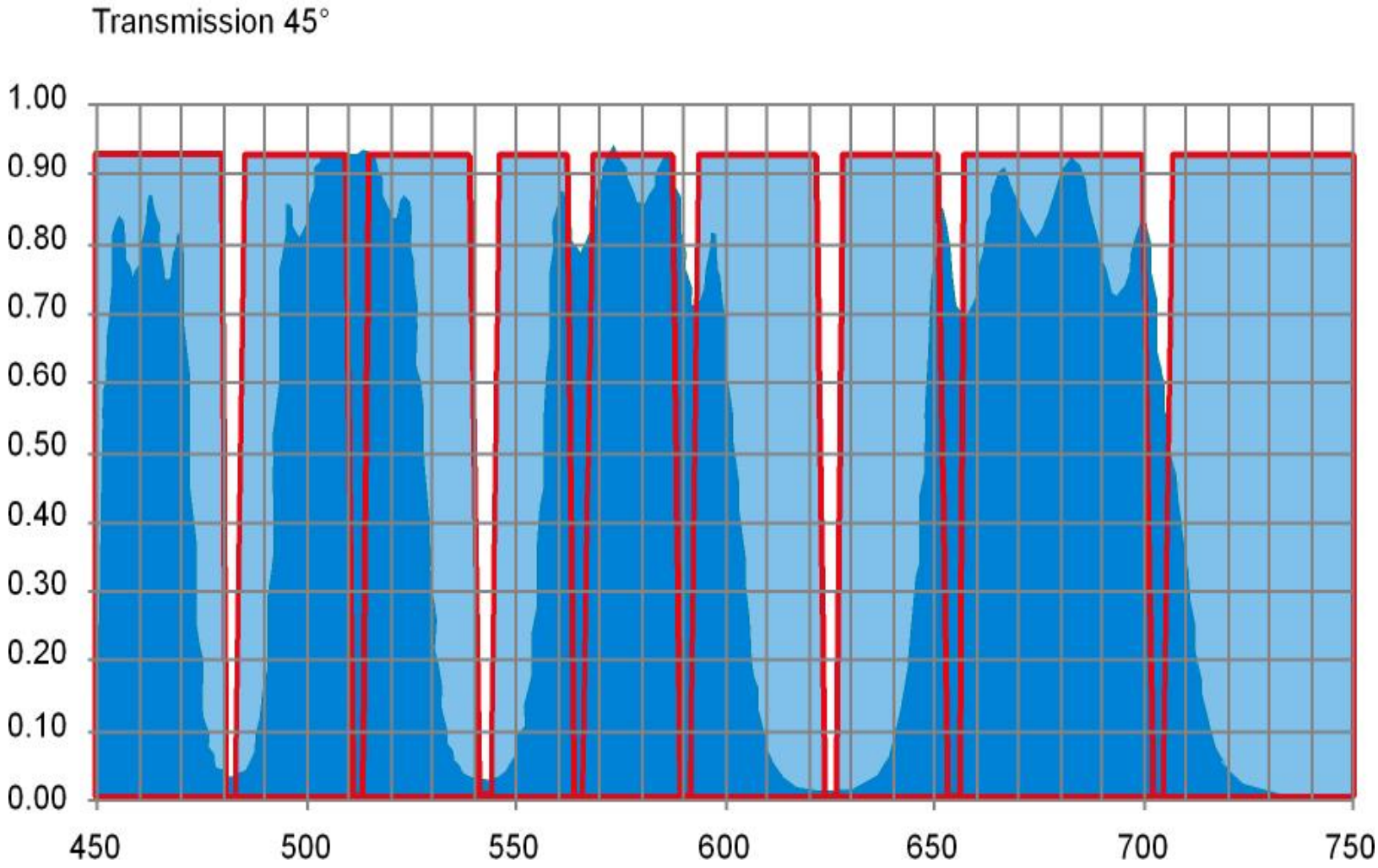
Leica AOBS:
More sensitive than beam splitters



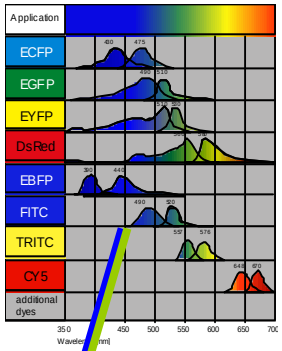


AOBS: More efficient

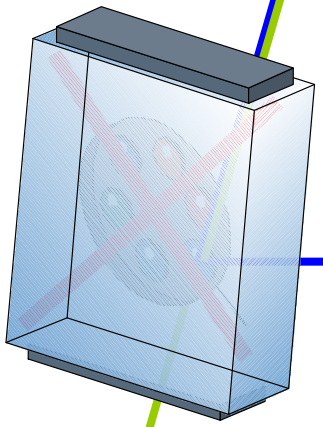
Triple Dichroic 488/543/633
(Chroma design curve)



programmable Beam-splitter!



AOBS, Leica 2002



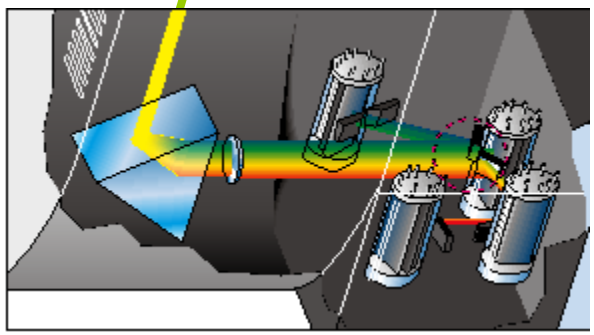
AOTF Leica 1993

AOTF | Light Source

standard:

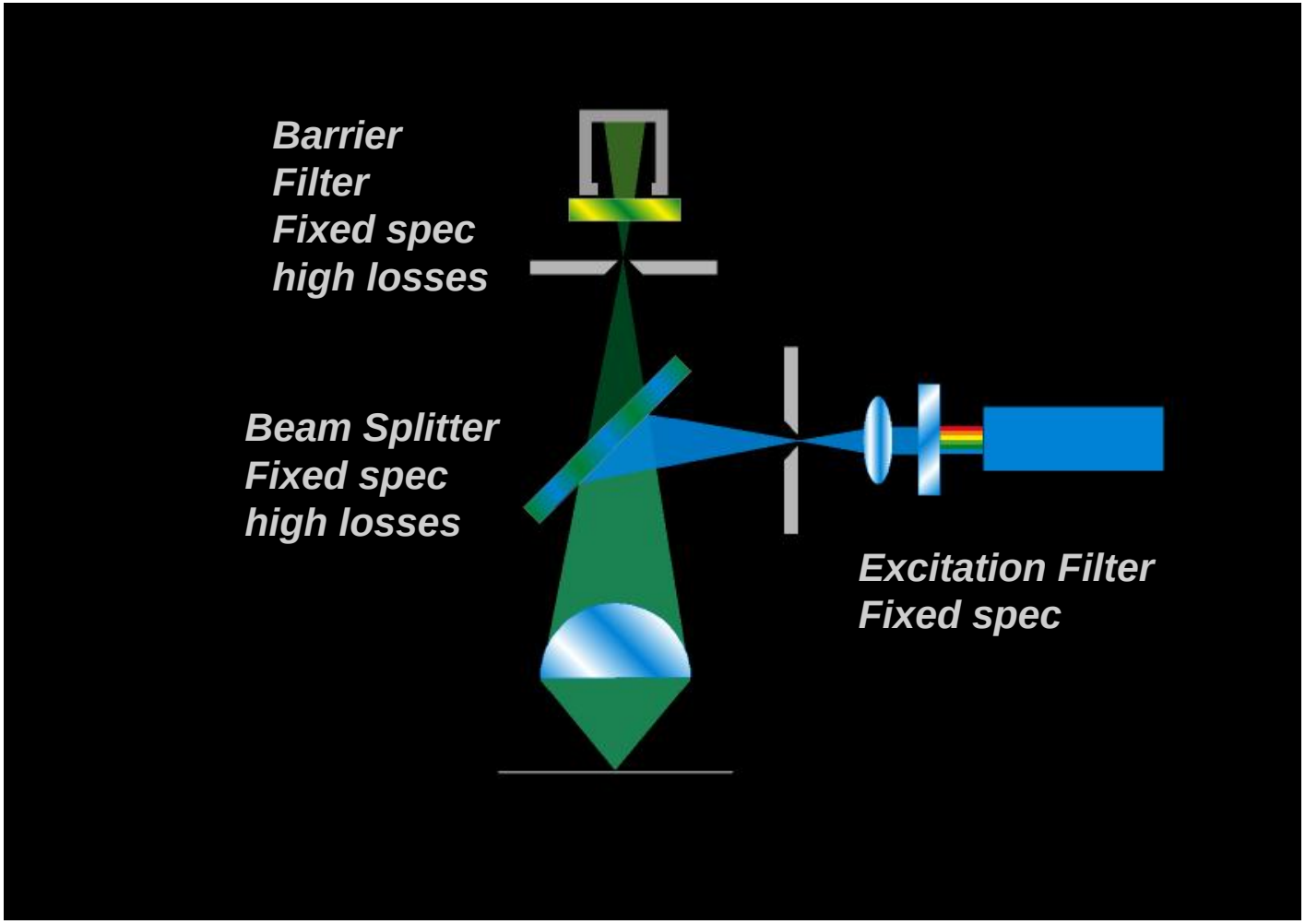
458 nm
476 nm
488 nm
496 nm
514 nm
543 nm
594 nm
633 nm

SP Leica 1997



Why AOBS?

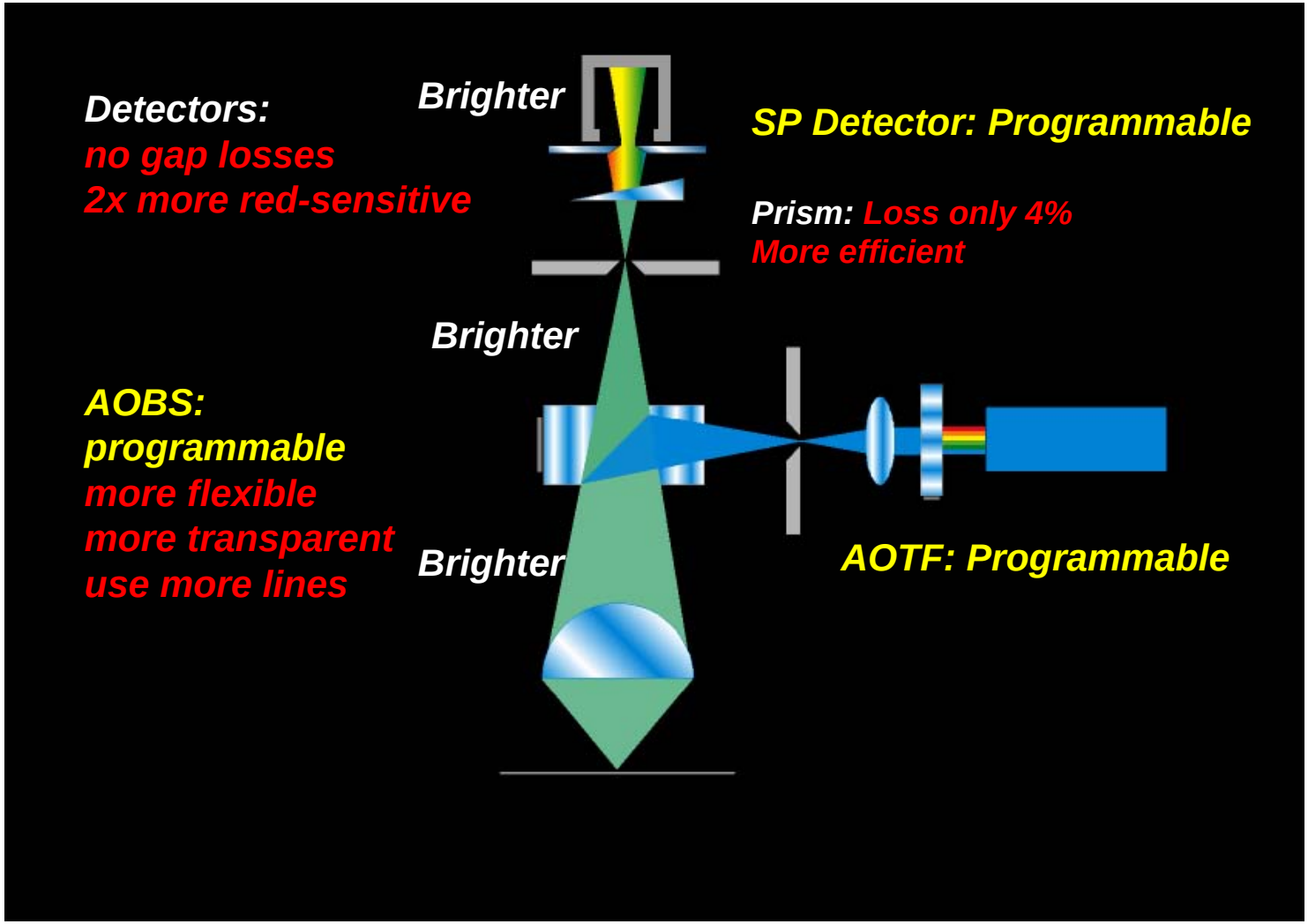
- Conventional Confocal Principle -



Why AOBS?

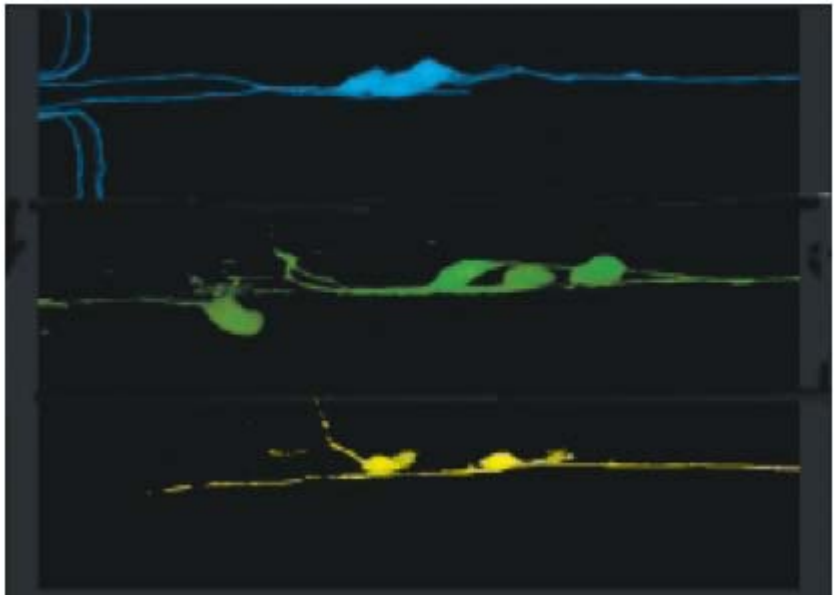
- LEICA TCS SP2/AOBS -

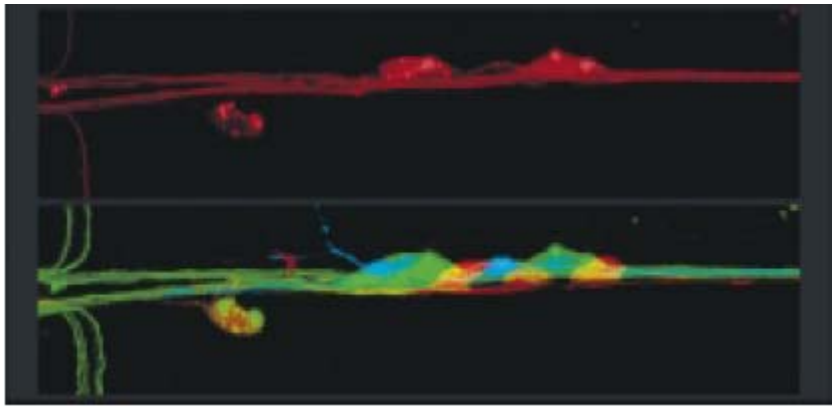
Leica MICROSYSTEMS



Powerful SP2 AOBS System

Leica
MICROSYSTEMS





C.elegans nervous system, seperation of 4 flourescent proteins: CFP, YFP, GFP, DS RED

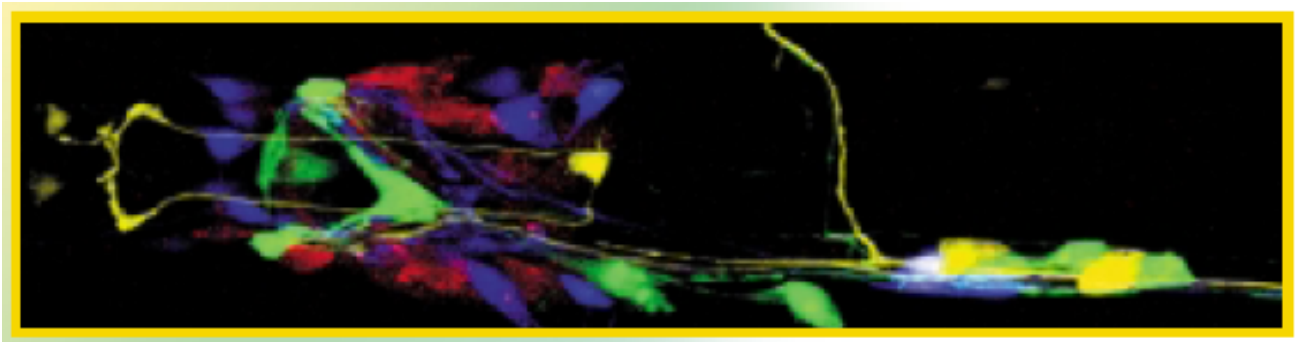
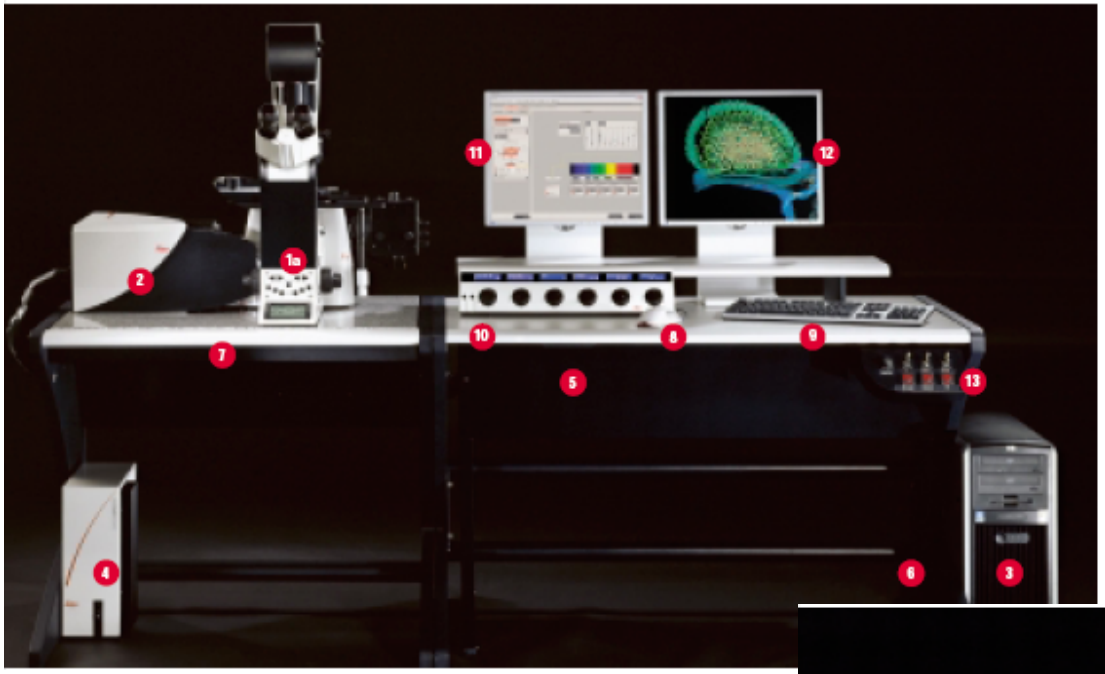


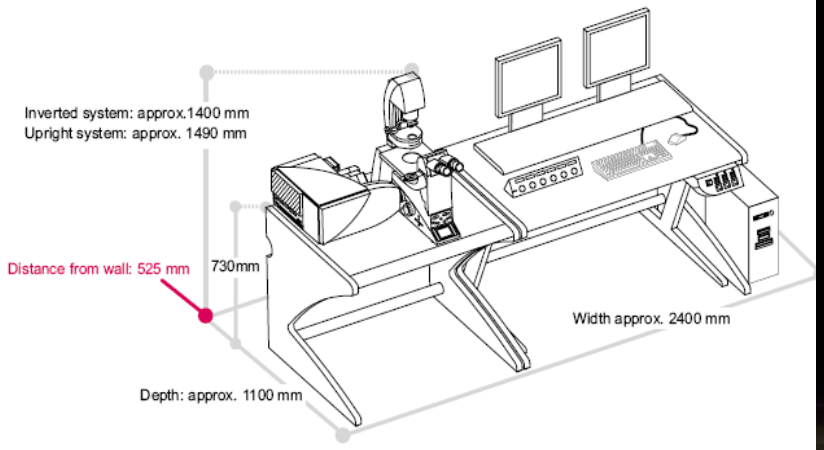
Fig. 1: C. elegans nervous system, (CFP, YFP, GFP, DS Red) acquired with a TCS SP2-AOBS.

LEICA TCS SP5 AOBS: *UV-VIS-MP*

Leica MICROSYSTEMS



1. Research Microscope
   a Inverted
   b Upright
2. Scanhead
3. Workstation
4. Microscope Control Unit
5. Laser and Power Supply
6. Computer Table
7. Microscope Table
8. Computer Mouse
9. Keyboard
10. Control Panel
11. Control Monitor
12. Image Monitor
13. Supply Control
14. Optical Table for Multiphoton Systems
15. Beam Routing for Multiphoton Laser
16. Multiphoton Laser

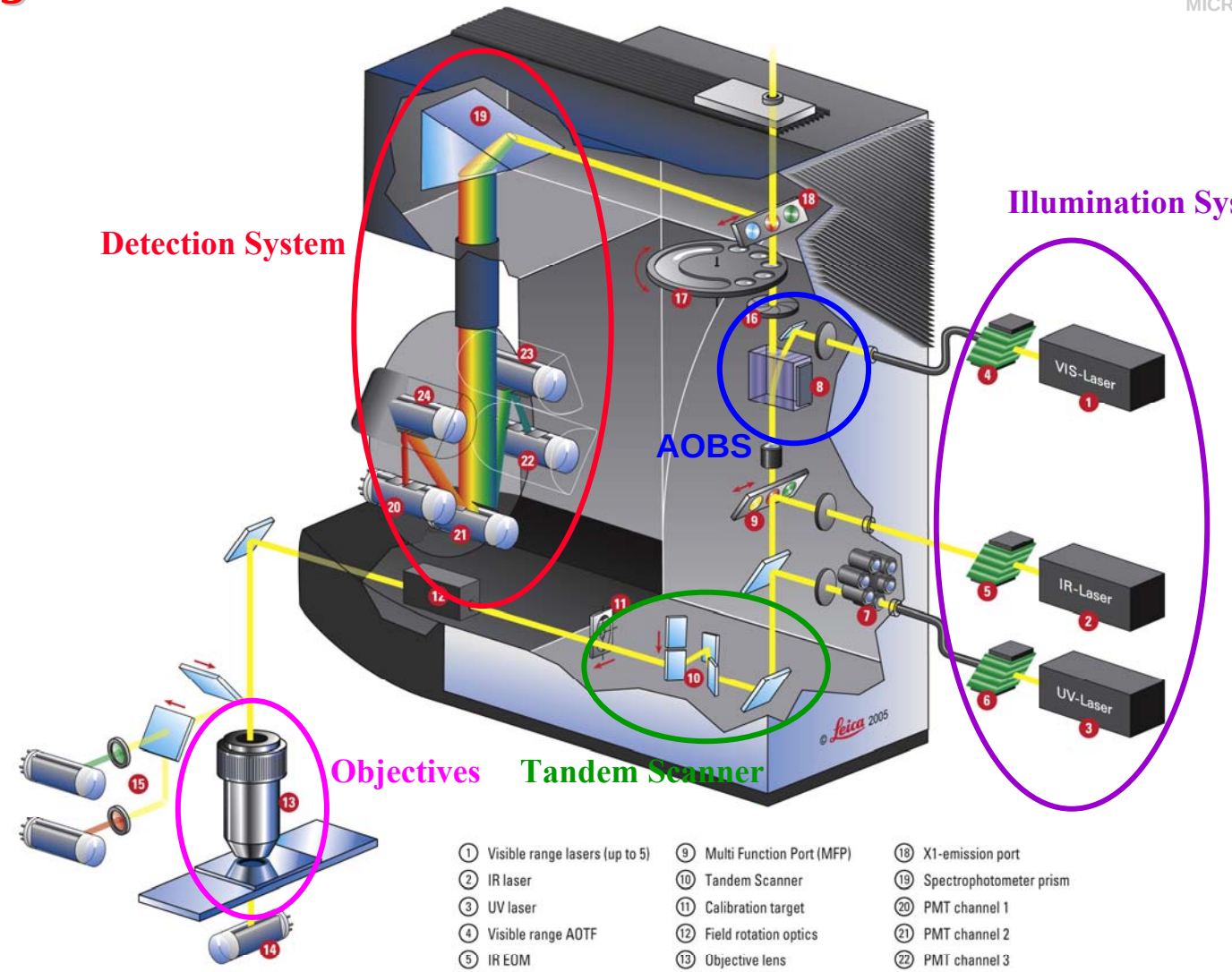


Major Instruments Co., Ltd. Taiwan

LEICA TCS SP5 Tandem System

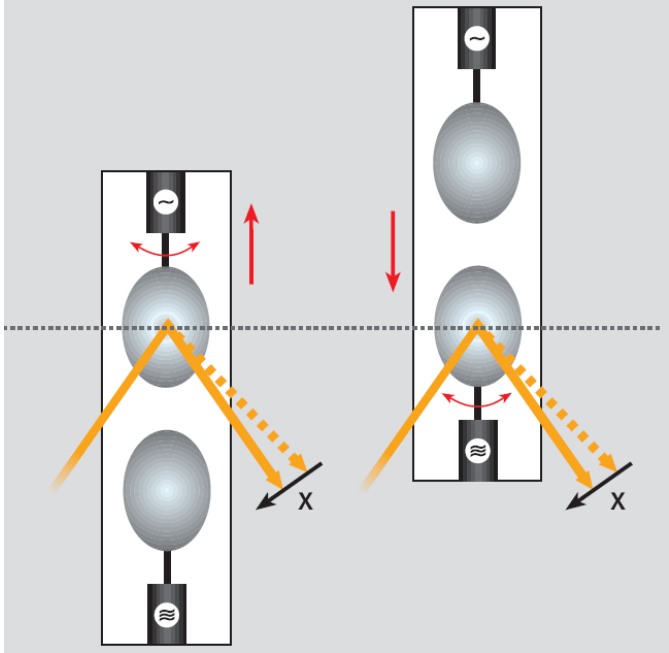
- Uniting two worlds -

Leica MICROSYSTEMS



① Visible range lasers (up to 5)
② IR laser
③ UV laser
④ Visible range AOTF
⑤ IR EOM
⑥ UV AOTF
⑦ UV optics imaging
⑧ AOBS
Acousto Optical Beam Splitter
⑨ Multi Function Port (MFP)
⑩ Tandem Scanner
⑪ Calibration target
⑫ Field rotation optics
⑬ Objective lens
⑭ Transmitted light dedector
⑮ Reflected light NDD
⑯ Confocal detection pinhole
⑰ Filter- and polarizer wheel
⑱ X1-emission port
⑲ Spectrophotometer prism
⑳ PMT channel 1
㉑ PMT channel 2
㉒ PMT channel 3
㉓ PMT channel 4 (optional FLIM)
㉔ PMT channel 5 (optional FLIM)

Leica TCS SP5:
Tandem scanner unites two worlds



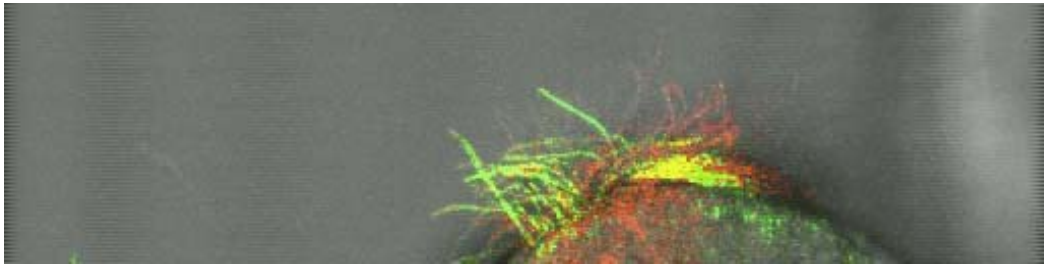
- *Two confocal systems in one scanner*
- *Resonant scanner for functional measurements at high speed*
- *Conventional scanner for spot recording and morphology*
- *Interchangeable, motorized*

Conventional mode – High resolution

Max. resolution: 8192 x 8192
Max. speed: 5 fps at 512 x 512
25 fps at 512 x 16

Resonent mode – High speed living cell

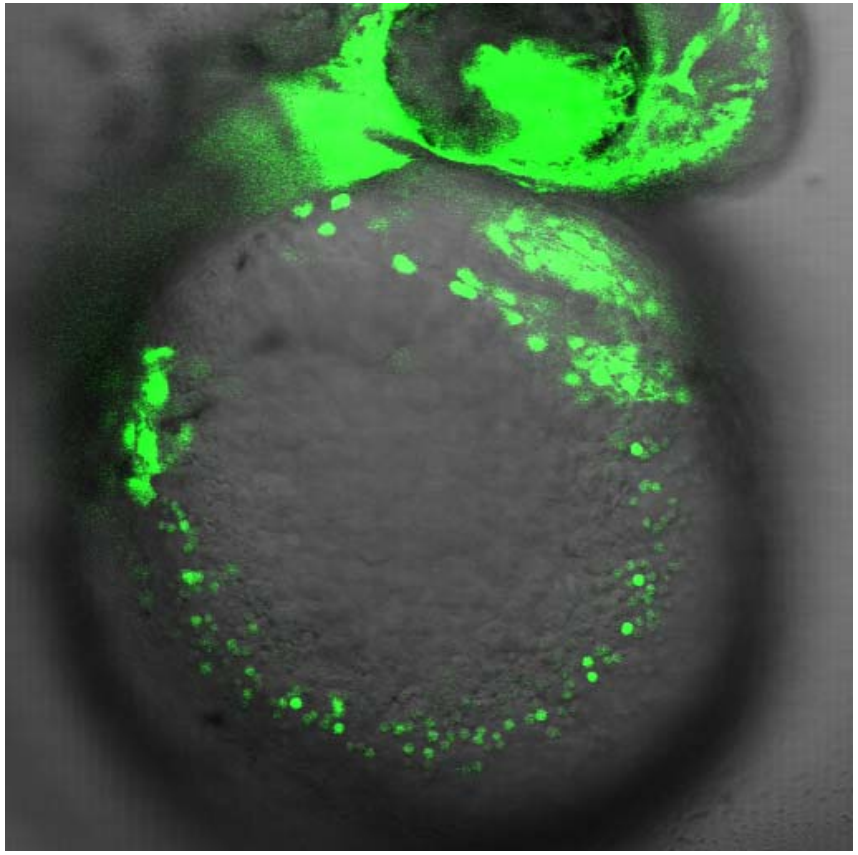
Max. resolution: 1024 x 1024
Max. speed: 25 fps at 512 x 512
250 fps at 512 x 16



LEICA TCS SP5 AOBS Tandem System

LEICA MICROSYSTEMS

- The Only Broadband Confocal Microscope -

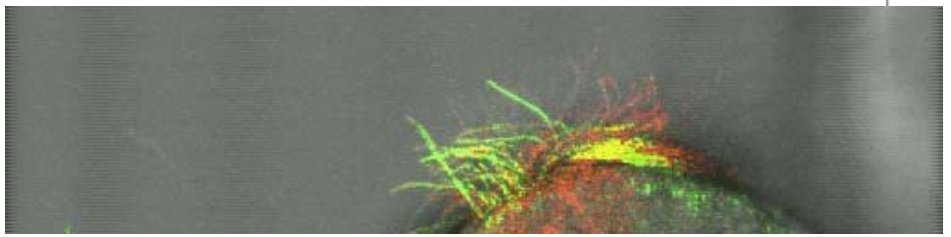


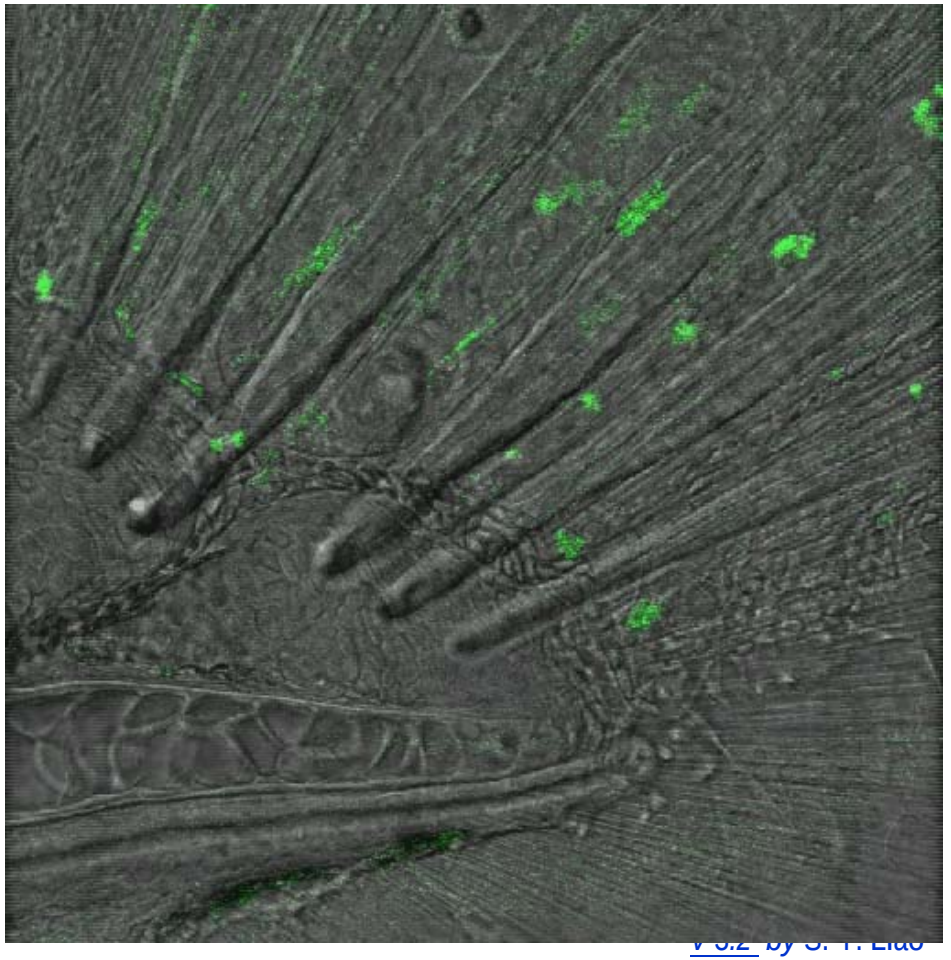
LEICA TCS SP5 AOBS Tandem - 中研院細生所 -

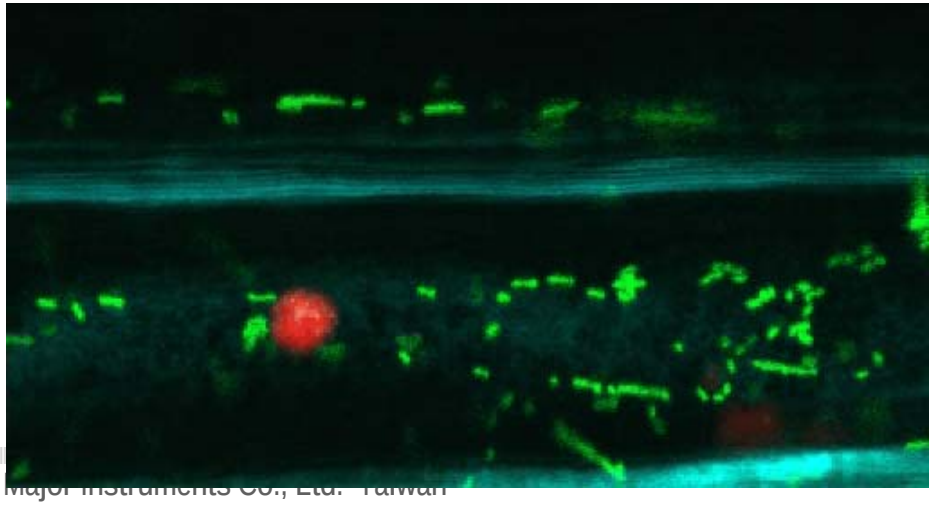
LEICA Confocal Systems in Taiwan

自1996年起至今，我們一共裝置了55套LEICA共軛焦系統。
其中TCS SP5共有19台，最高階的
TCS SP5 AOBS Tandem則有6台：

- 中央研究院 基因體中心
- 中央研究院 細生所
- 中央研究院 應用科學中心
- 陽明大學 微免所
- 台灣大學 生命科學院
- 台灣大學醫學院

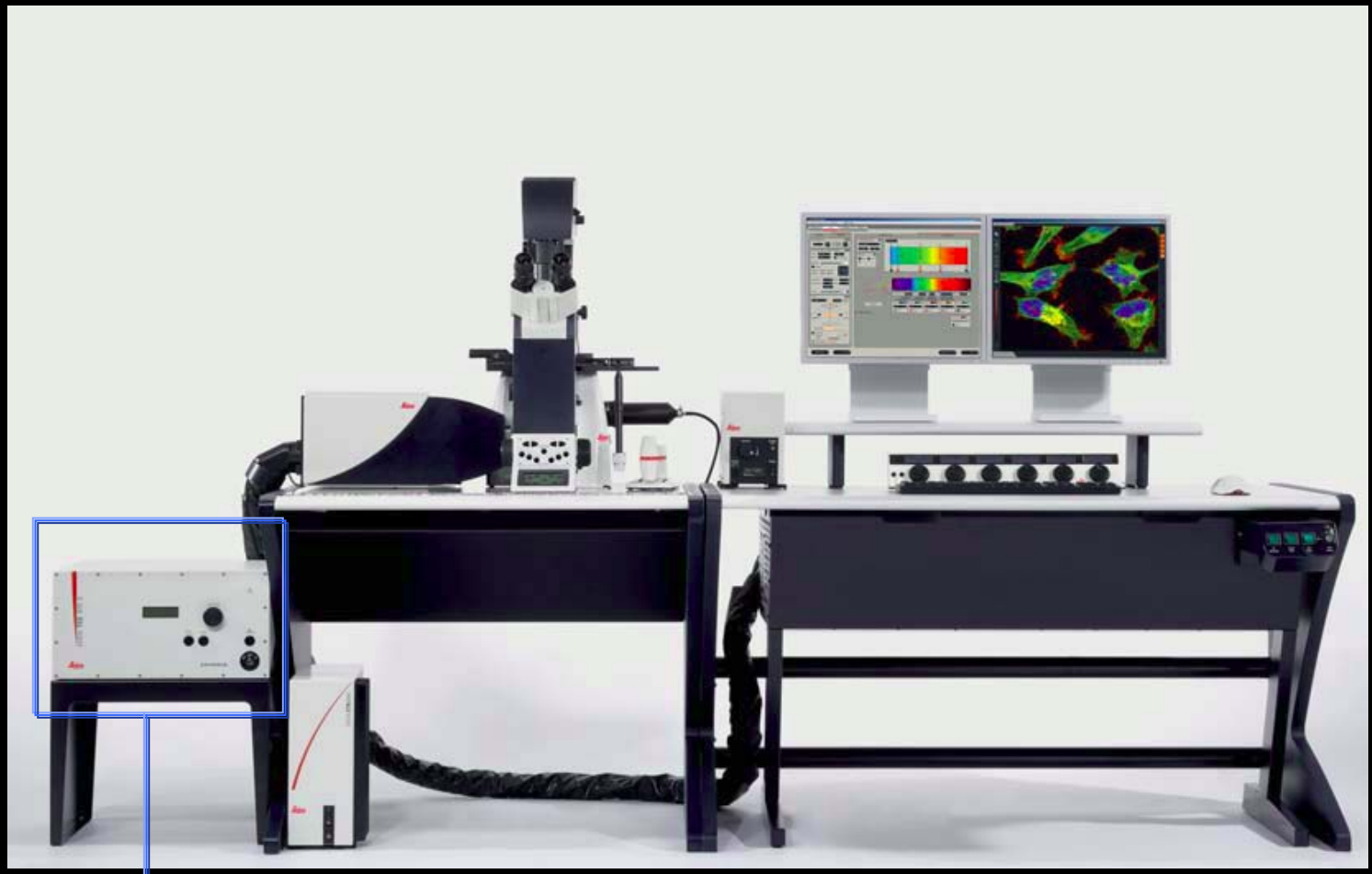






New Generation!

Leica TCS SP5 X AOBS Supercontinuum Confocal
- Tune into any excitation!

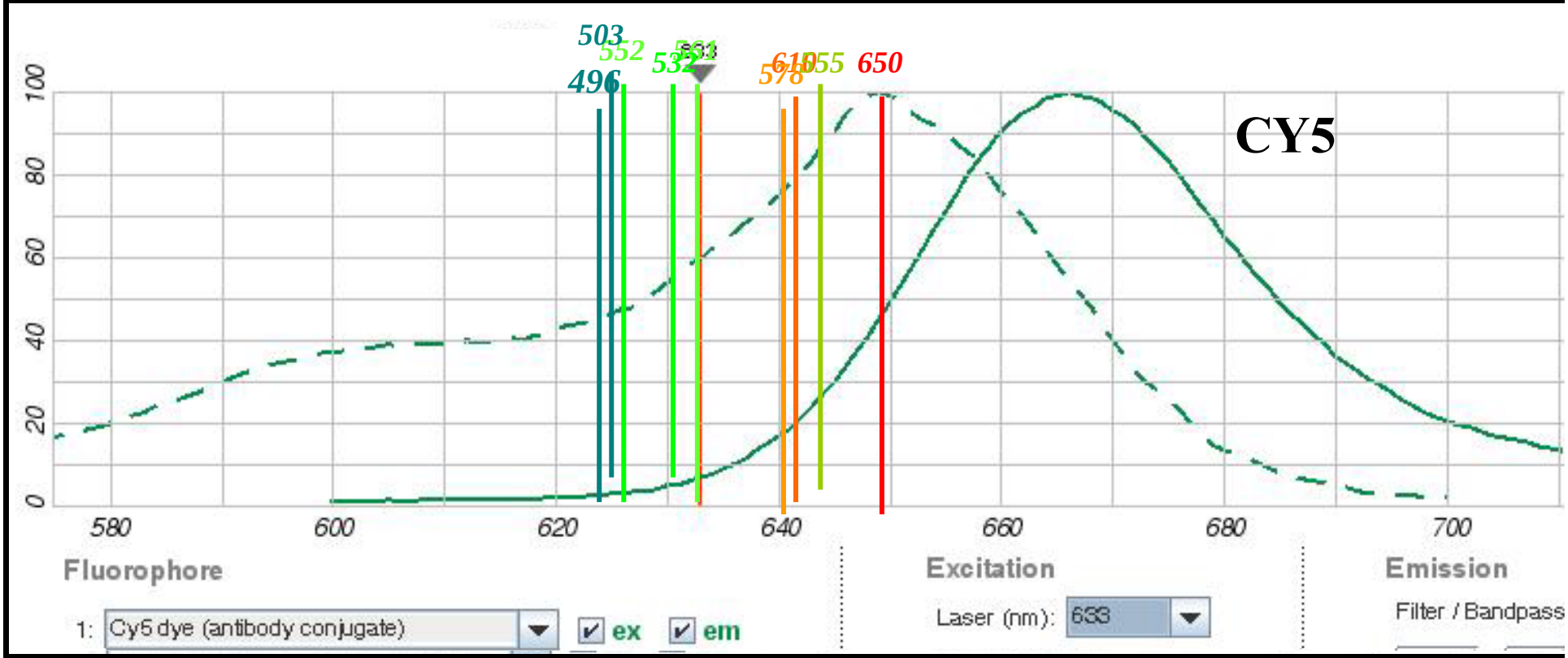


White light laser

TCS SP5 X AOBS

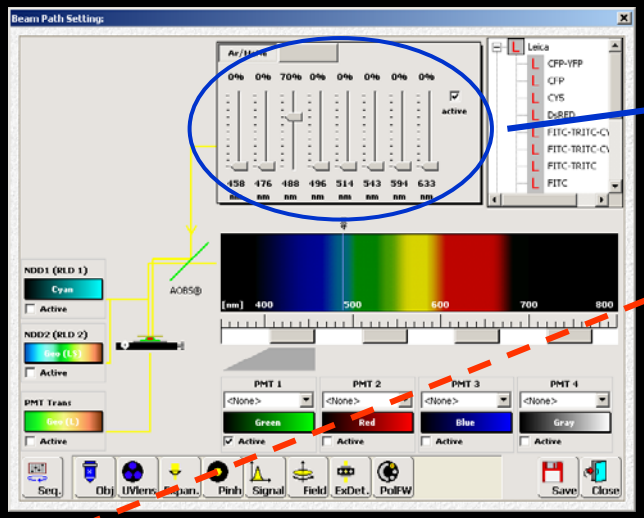
Excitation wavelength

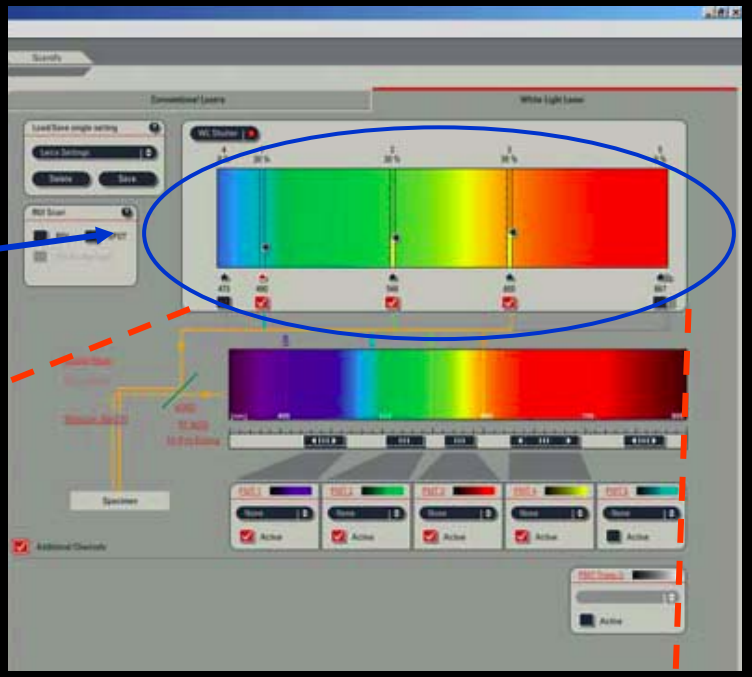
- 458nm, 476nm, 488nm, 496nm, 514nm, 561nm, 594nm, 633nm -

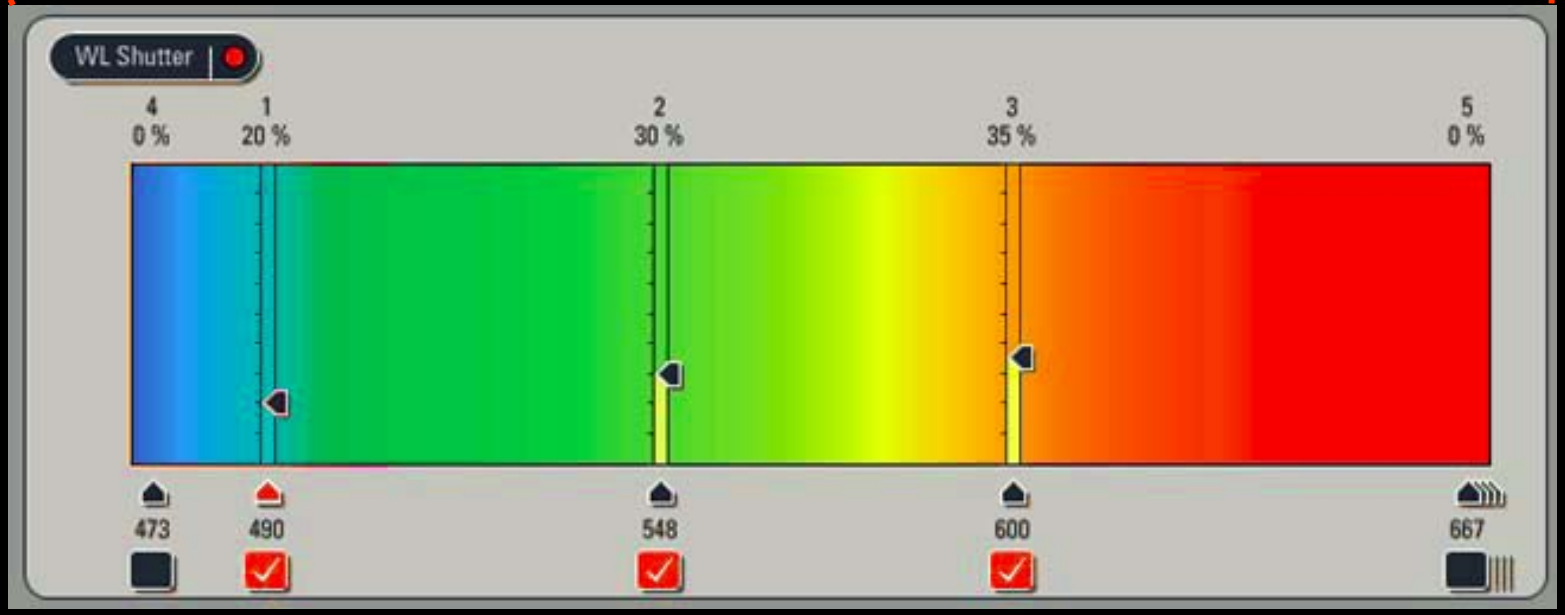


Fluorochrome	Excitation	Emission	Fluorochrome	Excitation	Emission
Alexa 488	495	519	Dil	549	565
Alexa 500	503	525	GFP(S65A)	471	504
Alexa 532	531	554	GFP(S65C)	479	507
Alexa 546	556	573	GFP(S65T)	488	511
Alexa 555	553	568	MitoT orange	551	576
Alexa 568	578	603	MitoT red	578	599
Alexa 594	590	617	NeuroT 500	500	525
Alexa 610	610	627	NeuroT 530	530	615
Alexa 633	621	639	NeuroT 640	640	660
Alexa 647	649	666	Rhodamine	550	573
Alexa 660	663	691	Rhod 123	507	529
Cy2	489	506	Rhod B	540	625
Cy3	552	570	Rhod Green	502	527
Cy3.5	581	598	Rhod Red	570	590
Cy5	649	670	Sytox green	504	523
Cy5.5	675	695	Sytox orange	547	570
DiD	644	665	Texas Red	595	620

AOBS unique advantage
- White Light Laser -

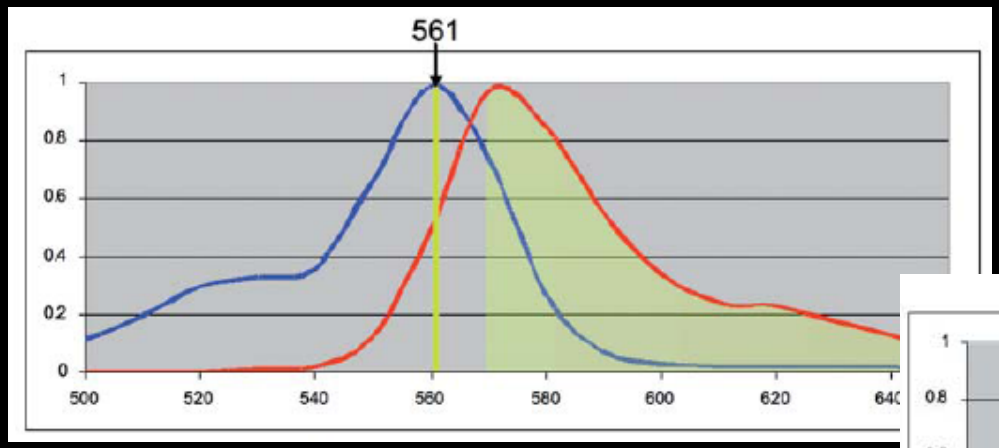


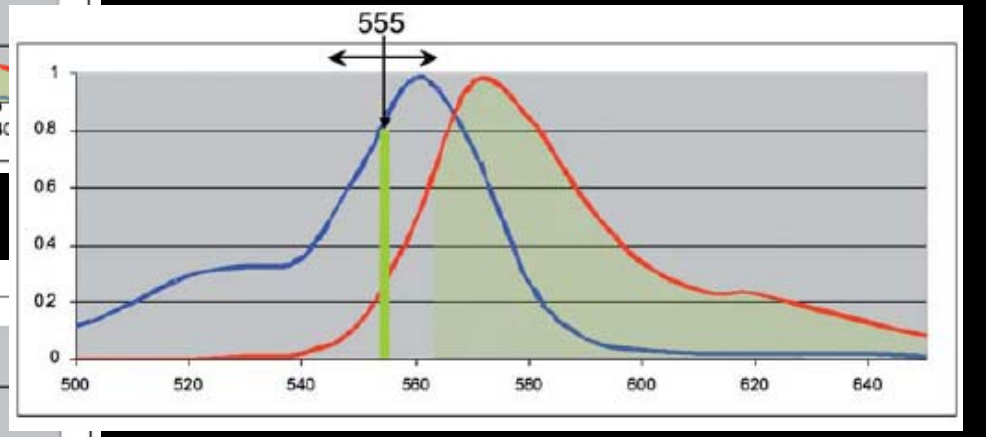


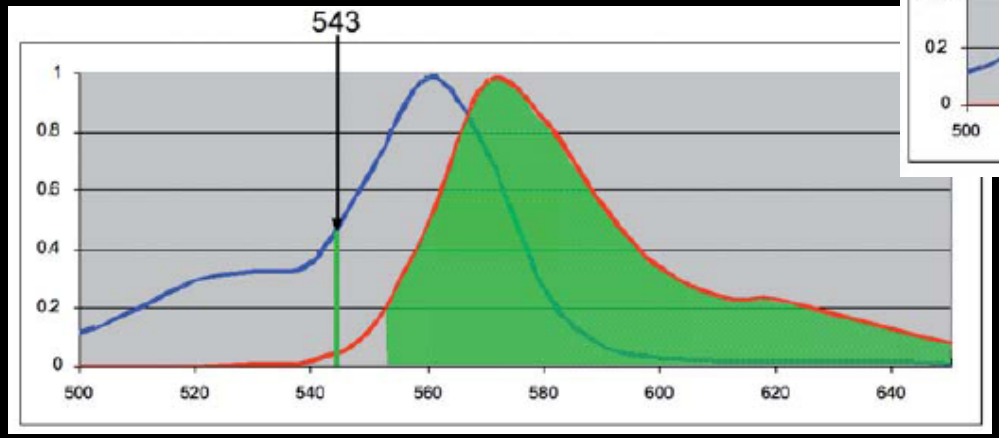


Benefit 1: Tune in to Exciation Optimum

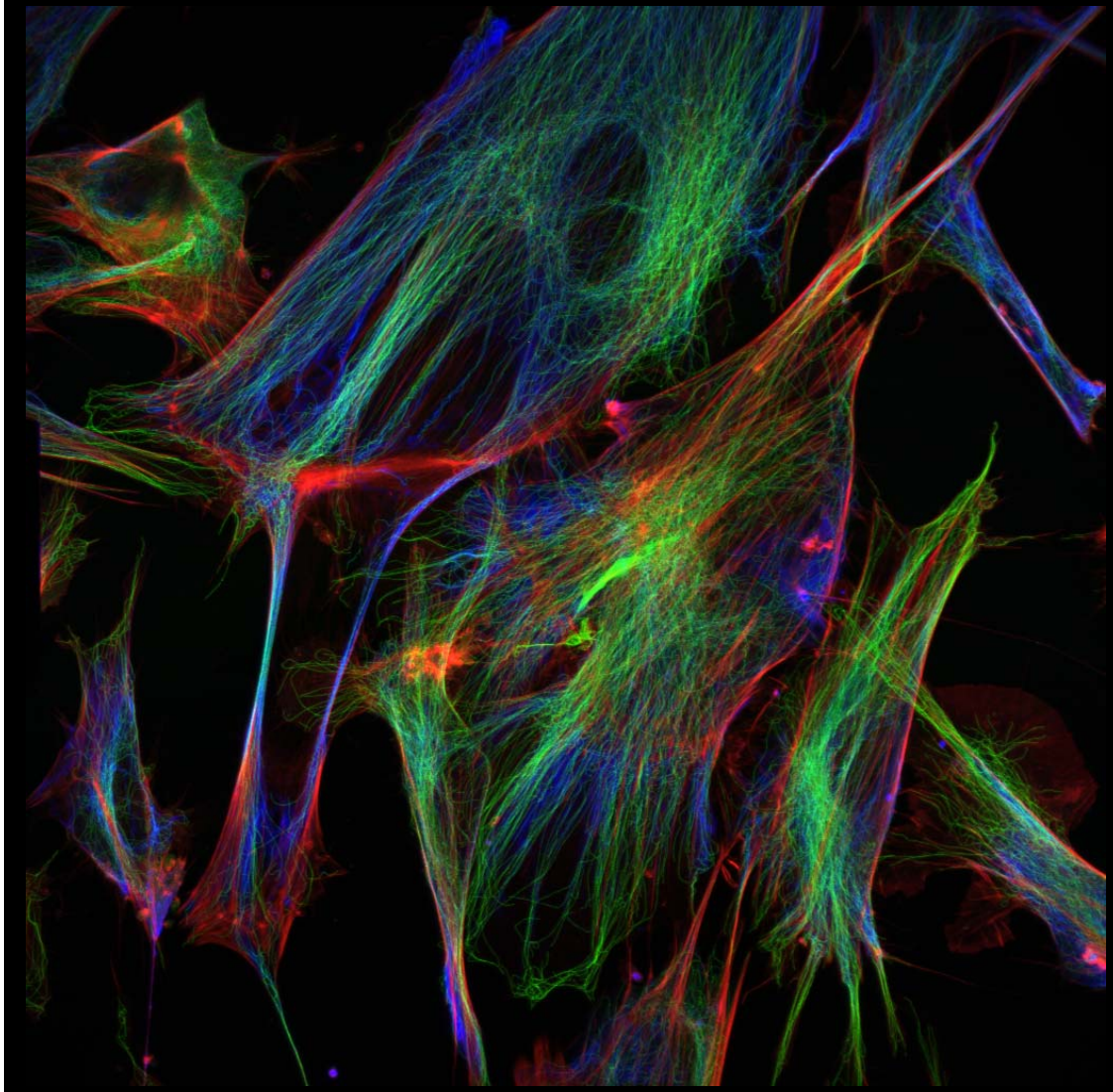
Example:Alexa 546





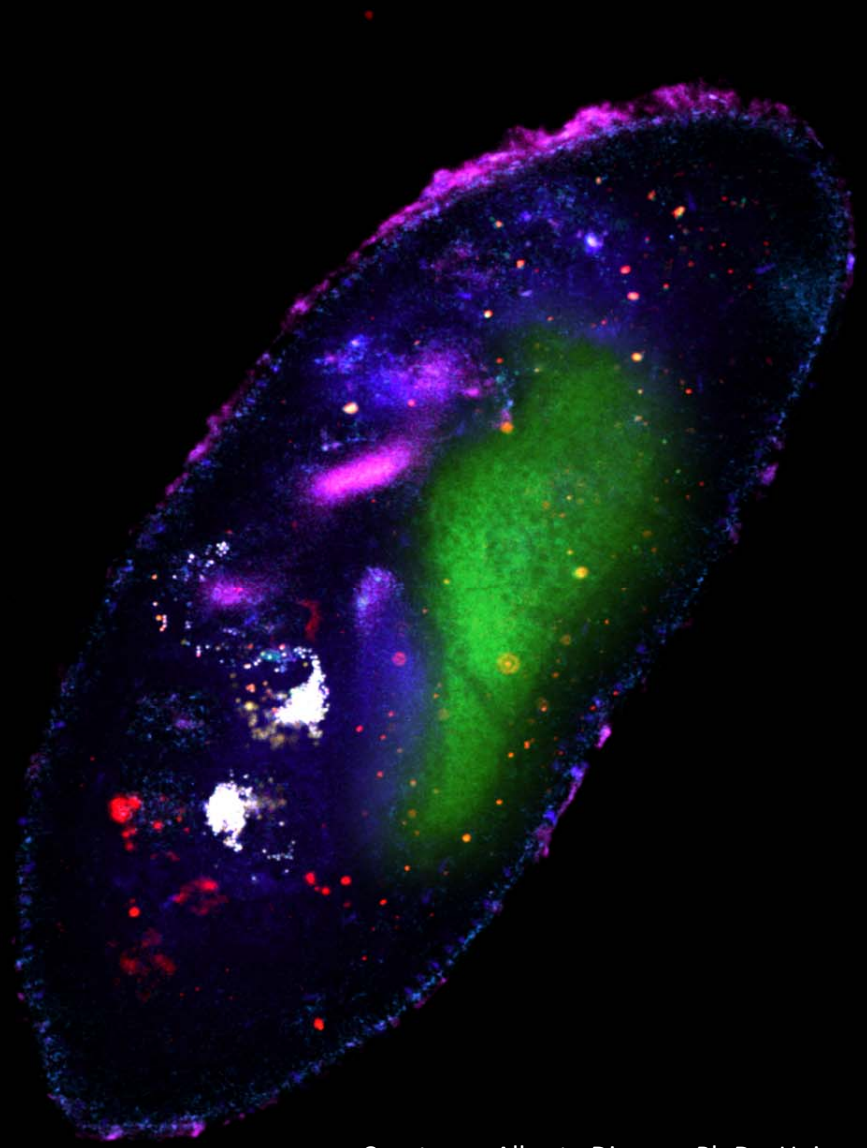


Low photo-damage imaging –
at new wavelengths and minimal laser power



Mouse Fibroblasts, triple staining		
Cytoskeletal Protein	**Excitation**	**Emission**
Microtubuli	492	494-556
F-Actin	560	566-632
Vimentin	646	659-800

White Light Laser excites 8 labels simultaneously



Courtesy : Alberto Diaspro, Ph.D. University of Genoa

CY2,
exc 491 nm, emis 499-505 nm
NMDA Receptors

Lp,
exc 474 nm , 509-514 nm,
beads in vacuols

Acridine Orange,
exc 484 nm, emis 520-532nm,
Nucleus

Cy3 ,
exc 551 nm, emis 555-575nm,
Gaba-B-R1 Receptors

TexasRed,
exc 562 nm, emis 605-612 nm,
Vacuoles and endosoms

ALEXA 594,
exc 594, emis 615-625nm,
nuclear and cellular membrane

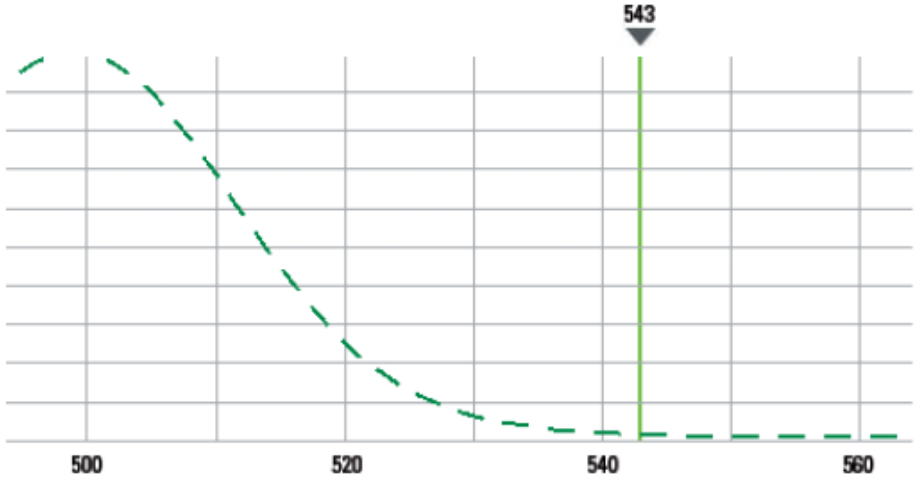
ALEXA 633,
exc 633nm, emis 655-665nm,
Clathrine vesicles

CY5,
exc 640 nm, emis 661-675nm,
Cilia

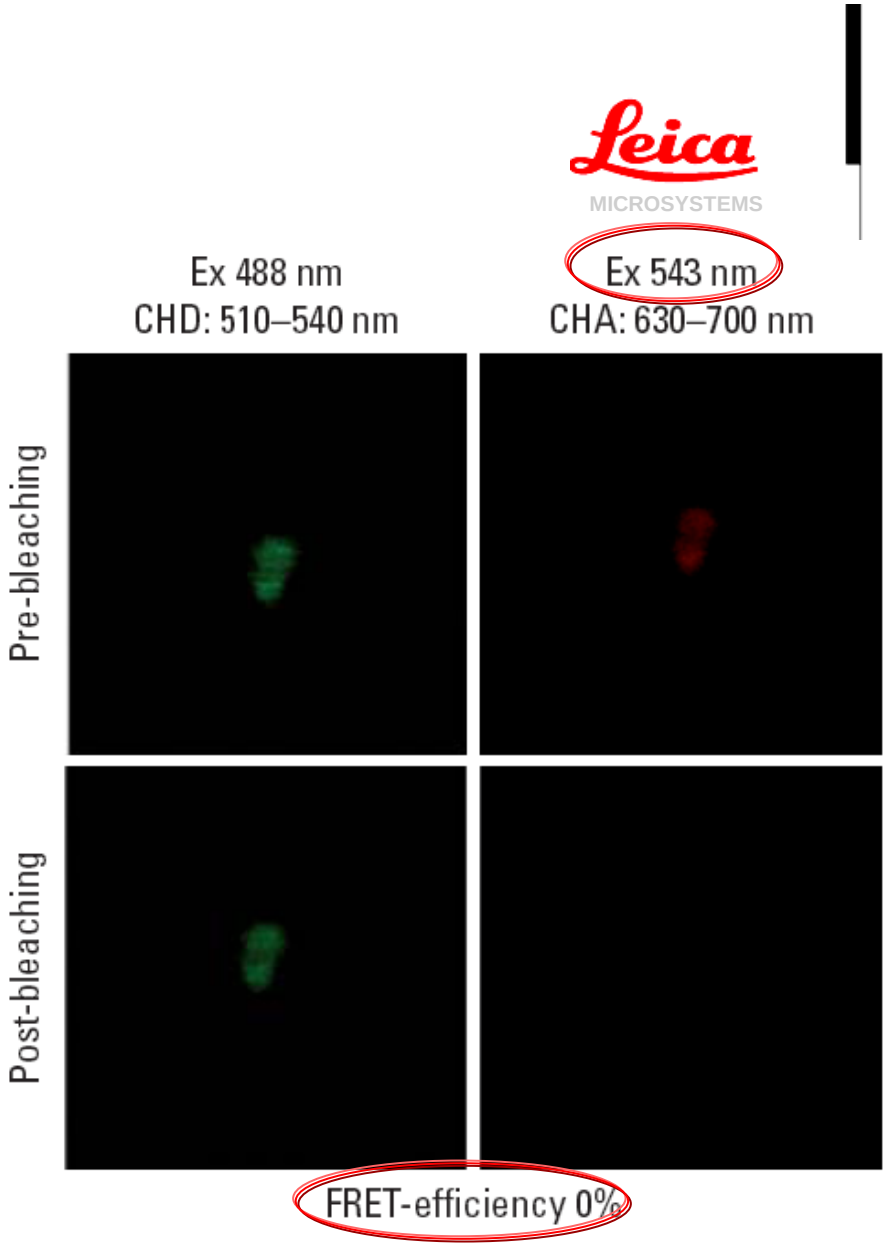
Benefit 2: FRET Optimized

FRET pair :Alexa 488 and Alexa 568

Donor excitation curve

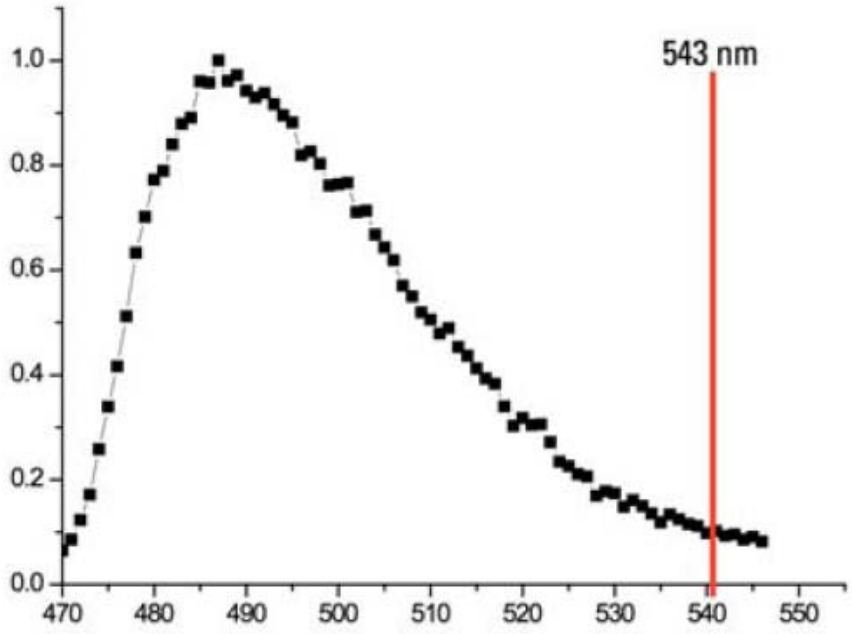


Invitrogen Spectra Viewer: Abs_{543} = 2%



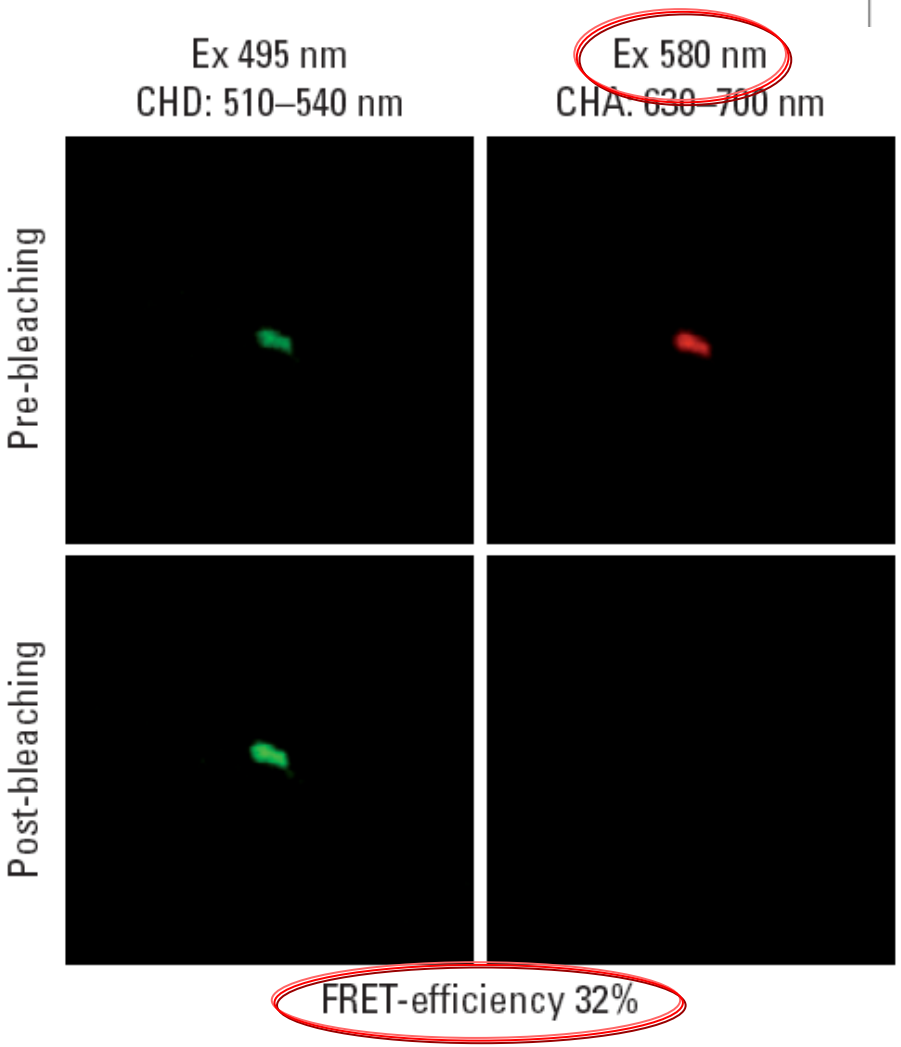
Benefit 2: FRET Optimized

Example:Alexa 488 and Alexa 568



Alexa 488 in white light laser
***In situ* Donor spectrum**

Donor *In-situ* Excitation Spectrum
Excitation at 543 = 10%



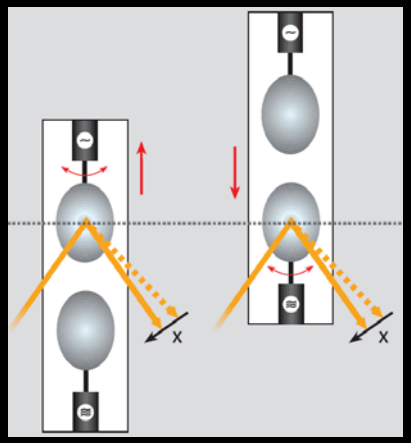
An Ideal Confocal System - Leica TCS SP5 X -

Whate Light Laser

Leica 2008

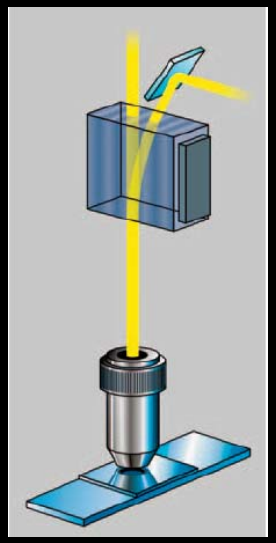
Tandem Scan

Leica 2005



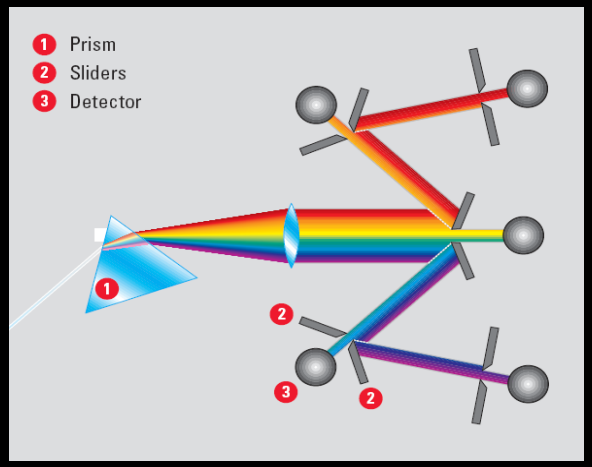
AOBS

Leica 2002



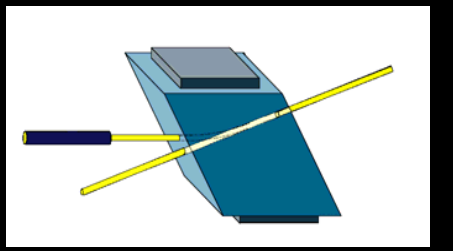
SP Detector

Leica 1997



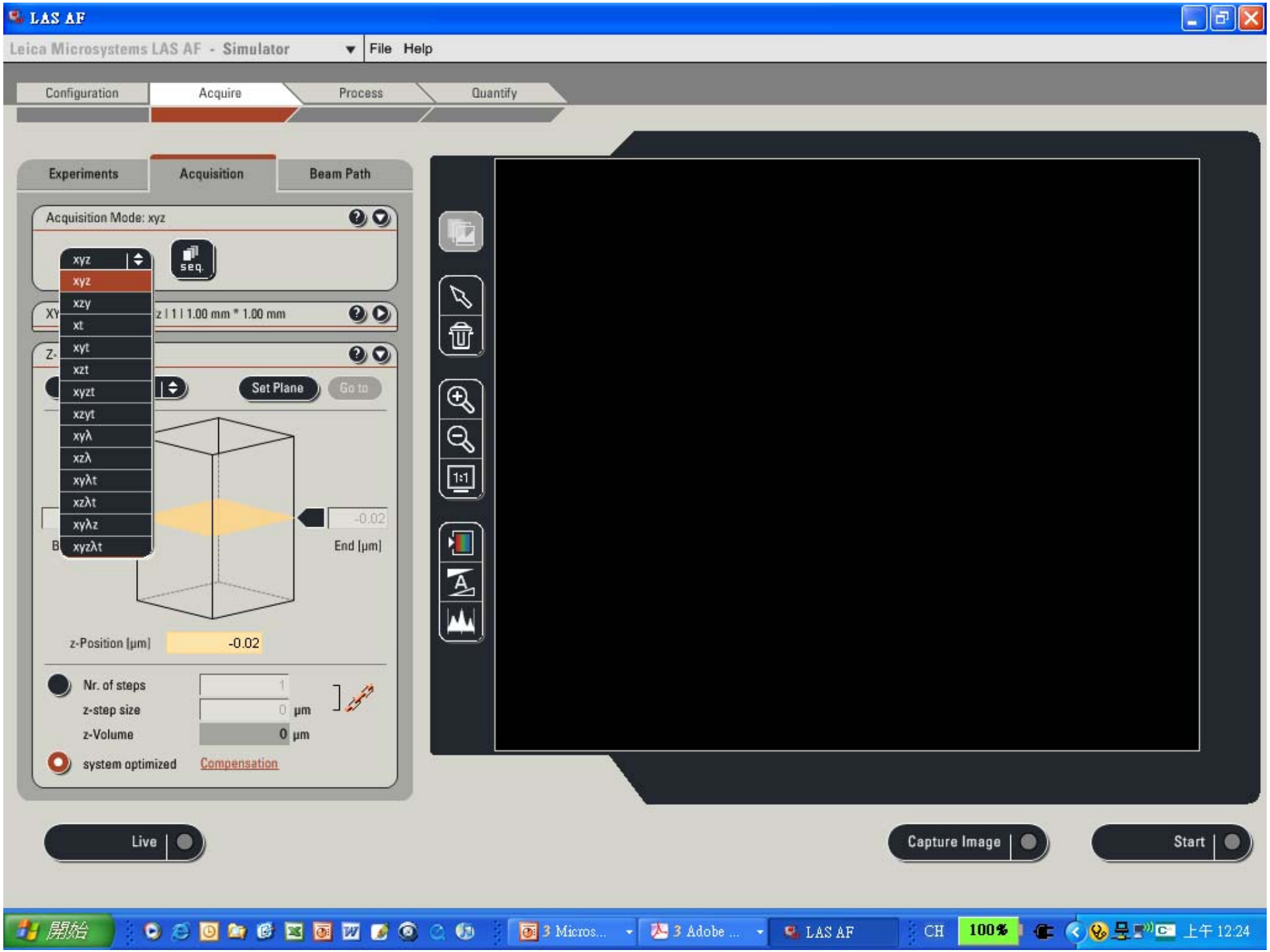
AOTF

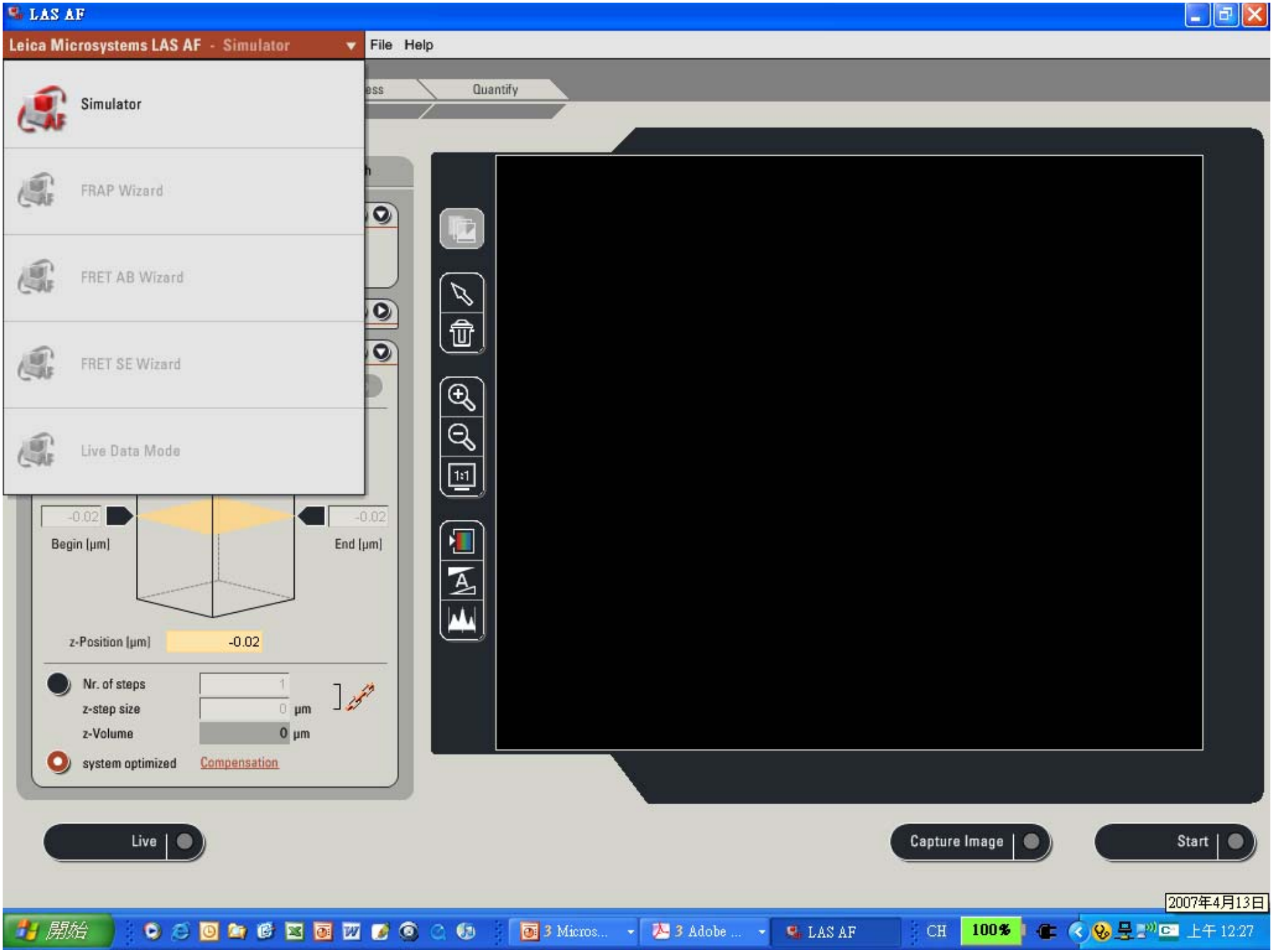
Leica 1993



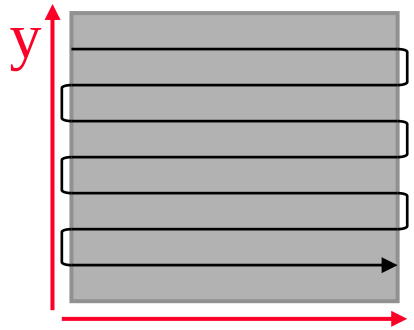
Leica
MICROSYSTEMS

Application


LAS AF
Leica Microsystems LAS AF - Simulator
File Help
Configuration
Acquire
Process
Quantify
Experiments
Acquisition
Beam Path
Acquisition Mode: xyz
xyz
seq.
xyz
xzy
xt
xyt
xzt
xyzt
xzyt
xyλ
xzλ
xyλt
xzλt
xyλz
xyzλt
z | 1 | 1.00 mm * 1.00 mm
Set Plane
Go to
-0.02
End [µm]
z-Position [µm]
-0.02
Nr. of steps
z-step size
µm
z-Volume
0 µm
system optimized
Compensation
1:1
Live
Capture Image
Start
開始
3 Micros...
3 Adobe ...
LAS AF
CH
100%
上午 12:24


LAS AF
Leica Microsystems LAS AF - Simulator
File Help
Simulator
FRAP Wizard
FRET AB Wizard
FRET SE Wizard
Live Data Mode
Quantify
-0.02
Begin [µm]
-0.02
End [µm]
z-Position [µm]
-0.02
Nr. of steps
z-step size
z-Volume
µm
system optimized
Compensation
Live
Capture Image
Start
2007年4月13日
開始
3 Micros...
3 Adobe ...
LAS AF
CH
100%
上午 12:27

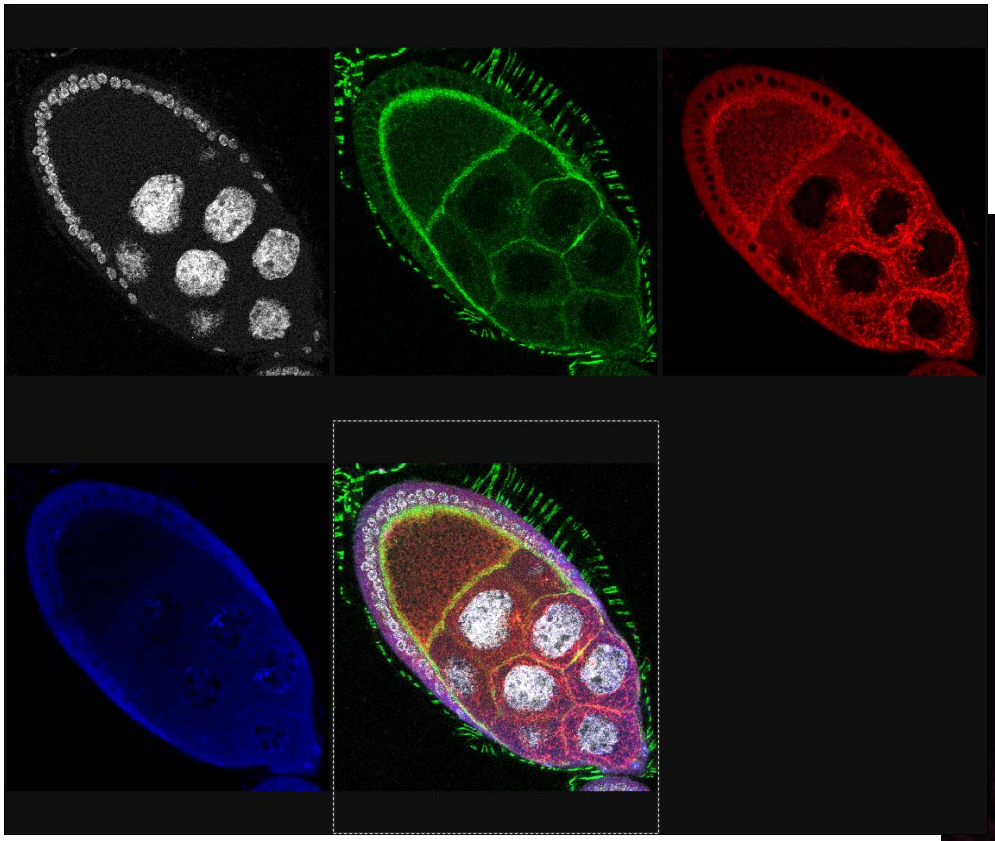
xy



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

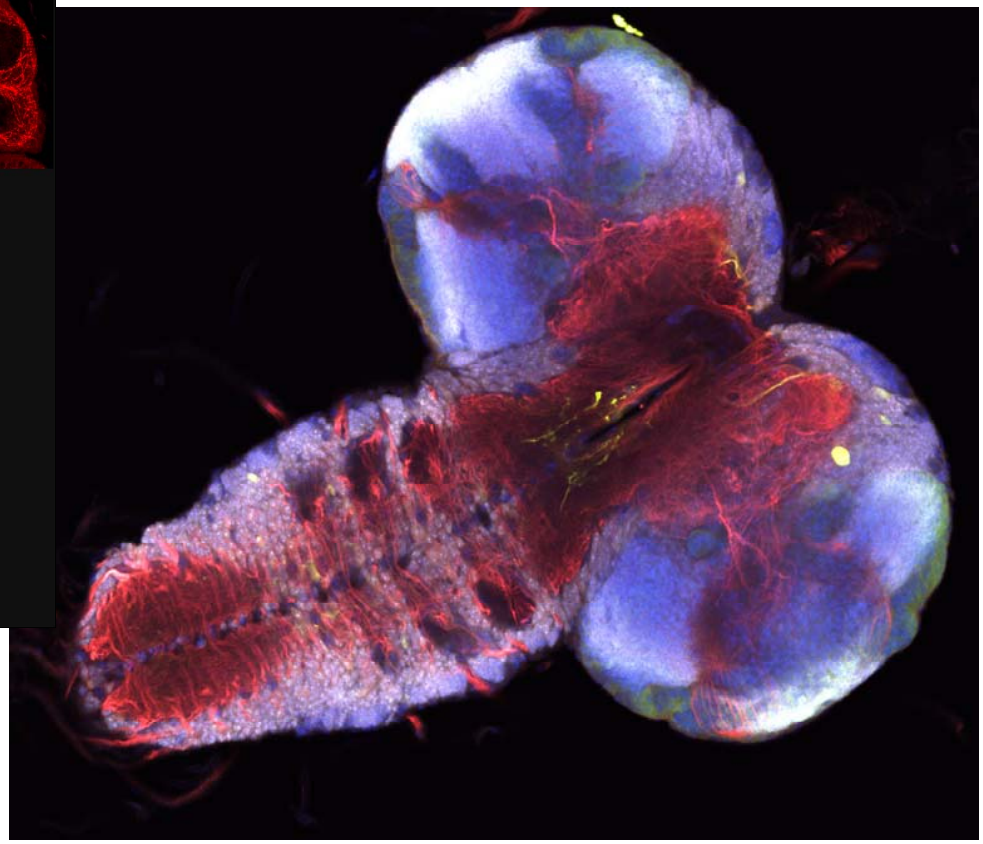
Scanning time depending onscan format (number of lines)

Leica MICROSYSTEMS

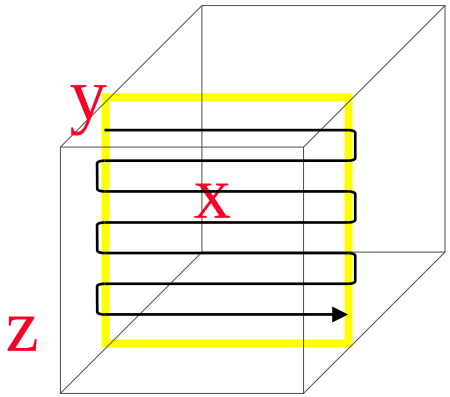


Drosophila melanogaster
Grey: Nuclei, DAPI
Green: Actin, Phalloidin
Red: XXX, Egalitarian
Blue: uncharacterized nuclear protein, Cy5
Courtesy Florence Besse & Oliver Hachet, EMBL, Heidelberg, Germany

Drosophila, 4k x 4k

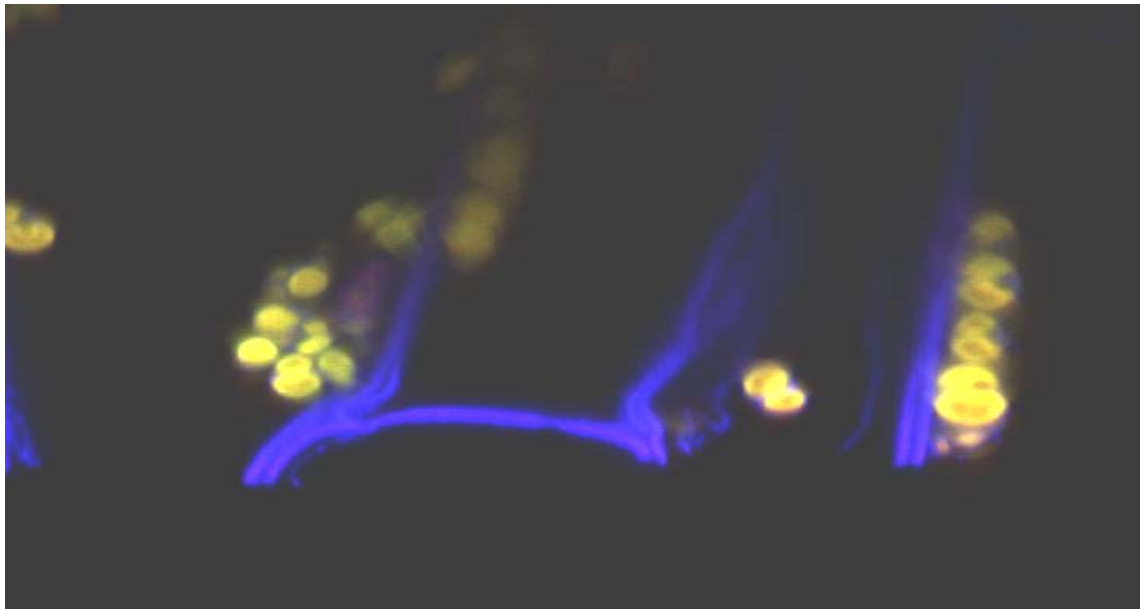


XZ



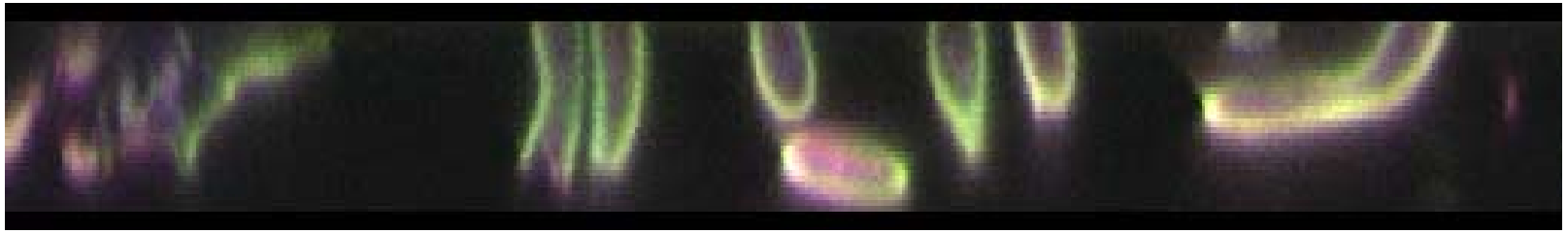
Z resolution depends on axial Resolution of objective, generally 2x less than in xy
xy: 180 nm, z: 360 nm

Leica MICROSYSTEMS

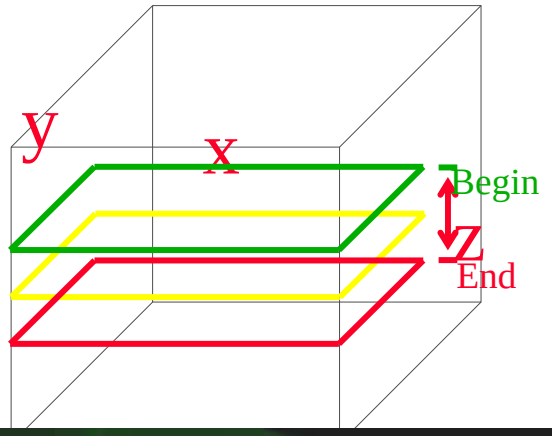


Convallaria

Starch grain
Cell wall

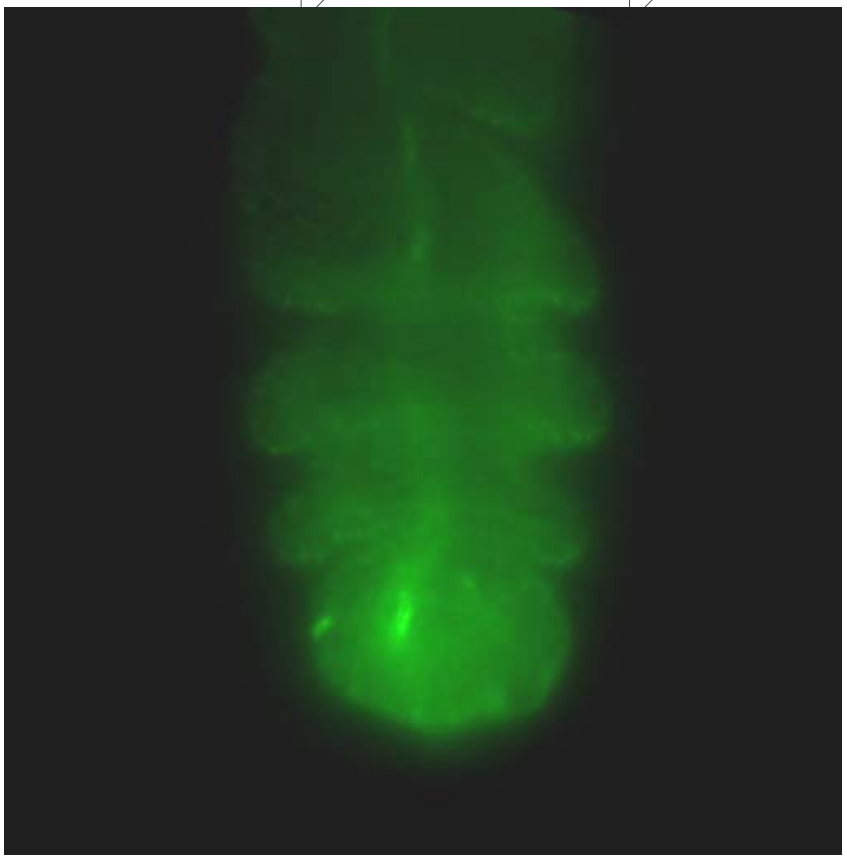


xyz

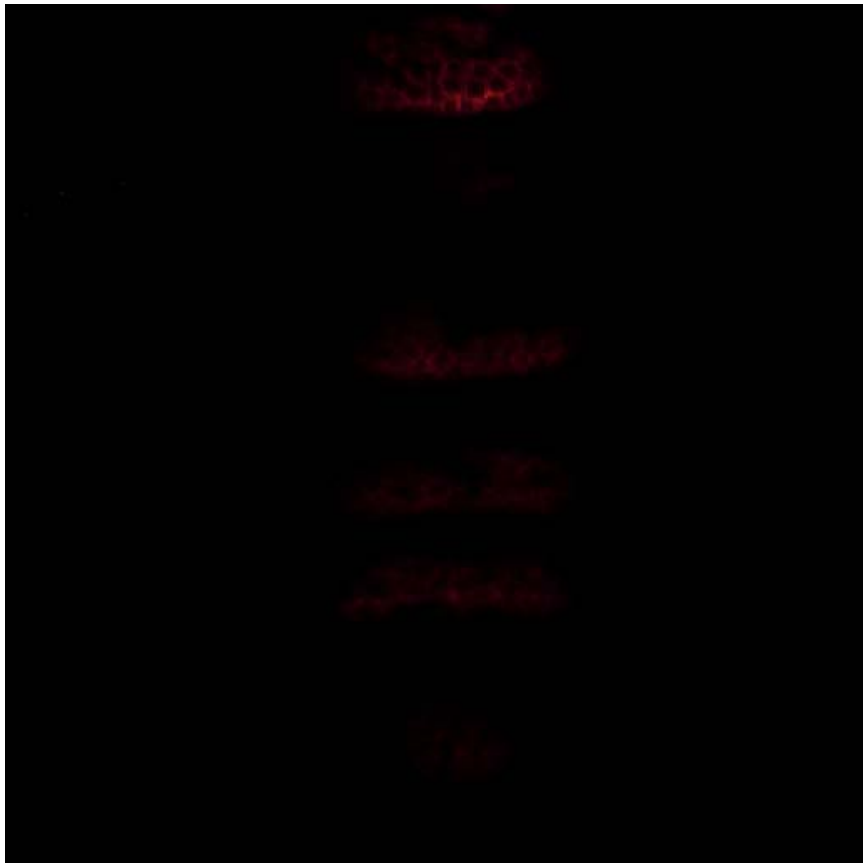


Beam is scanned
in x and y direction
and sample is moved
in z via galvo stage or electronic
focus of microscope

Leica MICROSYSTEMS

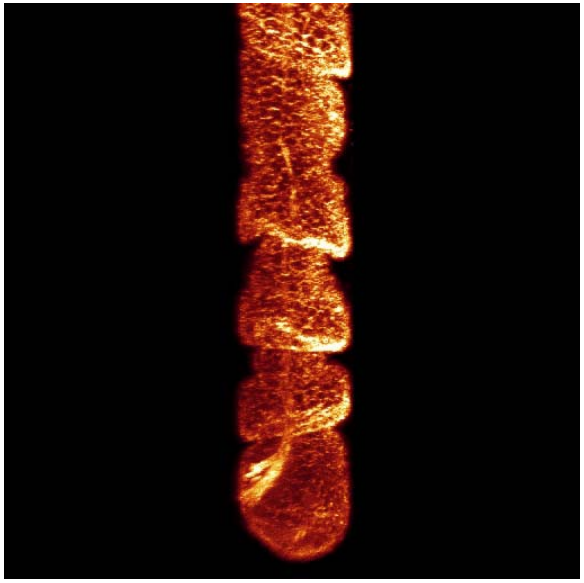


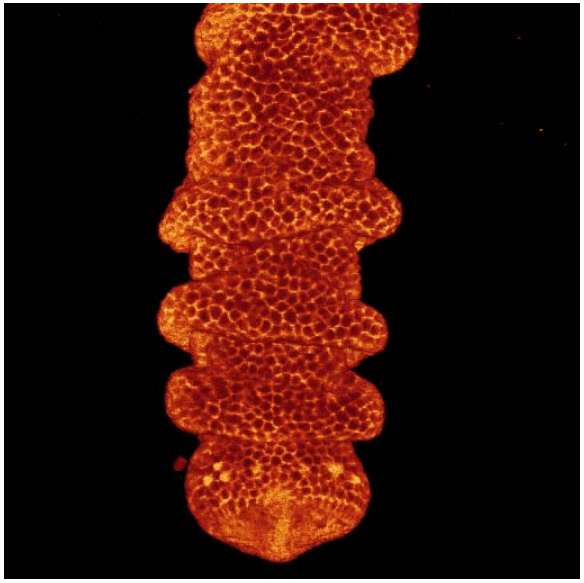
Drosophila leg, FITC, non-confocal

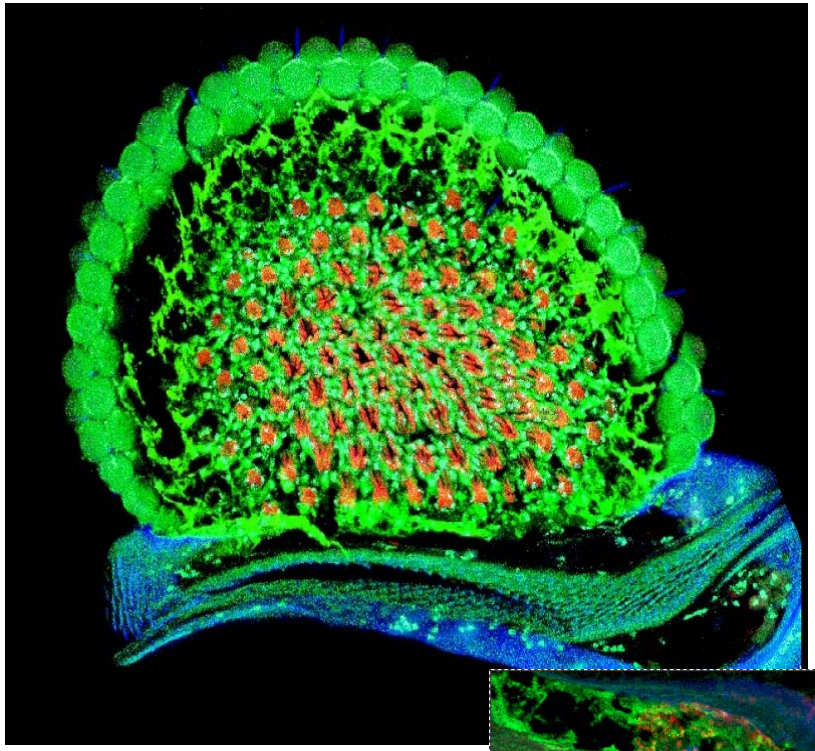


confocal

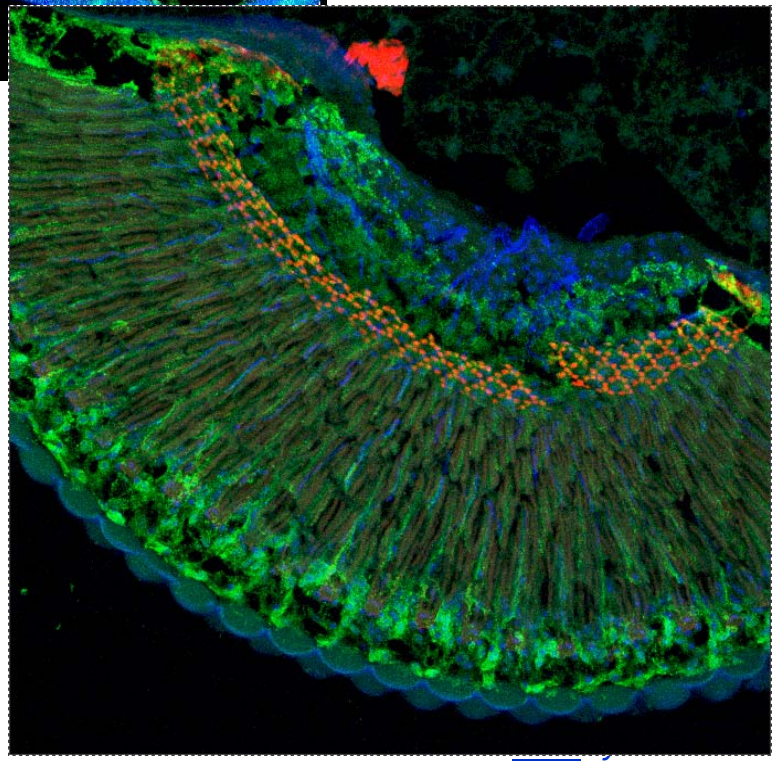
xyz





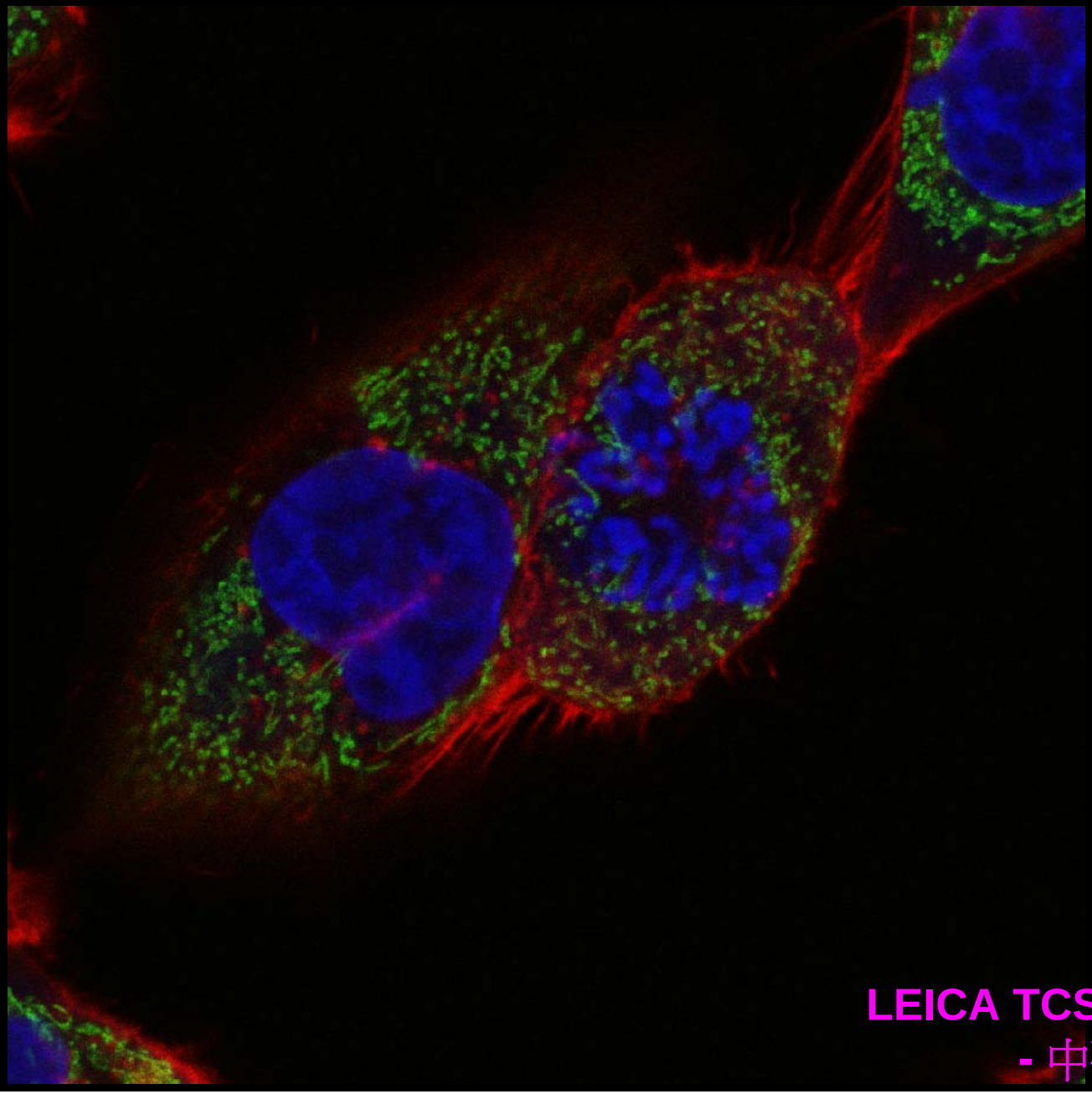


Drosophila melanogaster, eye section
Red: F-Actin, Cy3
Blue: Nuclei, DAPI
Green: pigmented cells, GFP
Courtesy of Anne Galy, IGBMC,
Strasbourg-Illkirch, France



Drosophila melanogatser, eye section
Red: F-Actin, Cy3
Blue: Nuclei, DAPI,
Green, Retina cells, GFP
Courtesy of Anne Galy, IGBMC,
Strasbourg-Illkirch, France

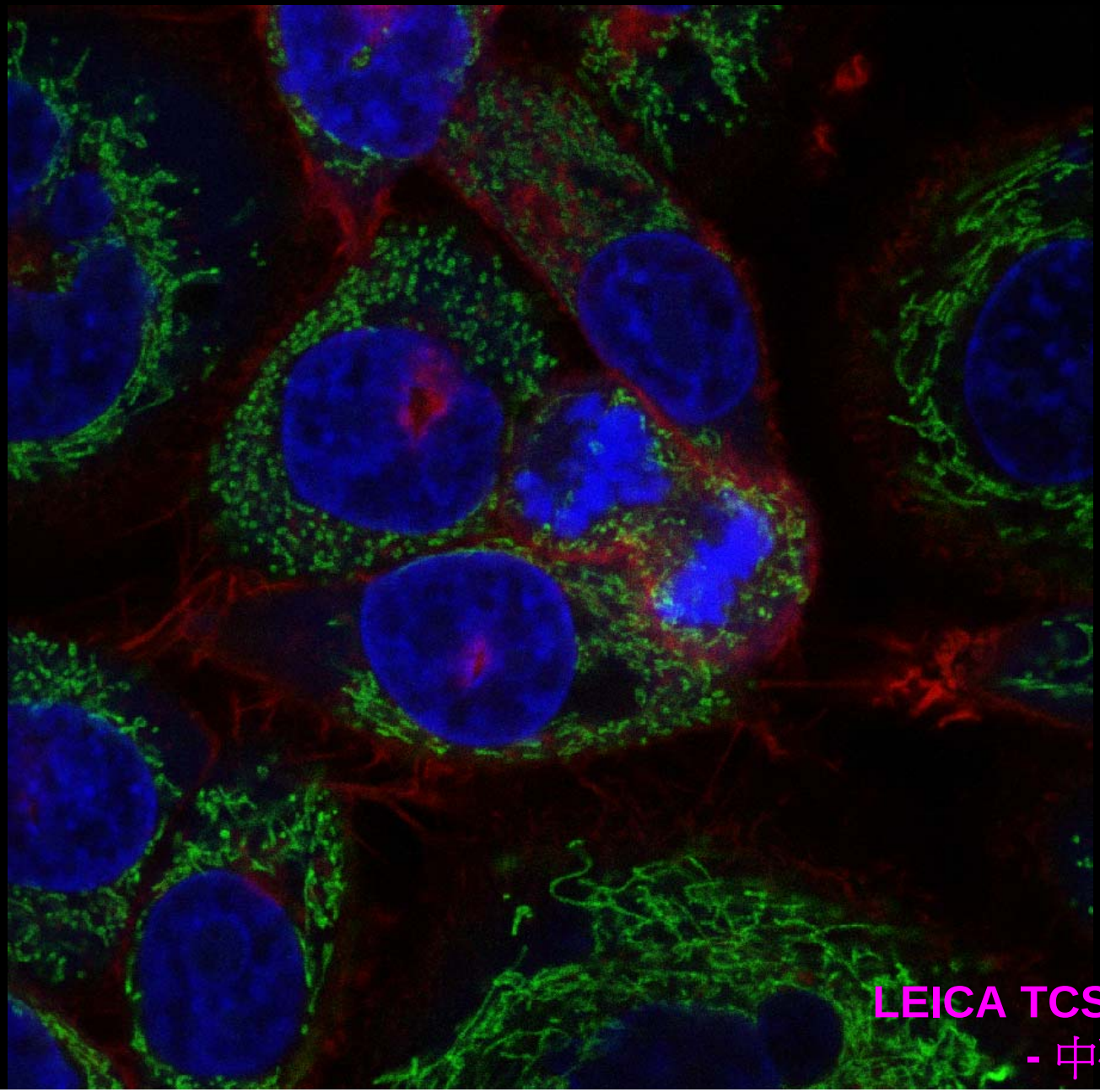
XYZ projection



LEICA TCS SP5 AOBS

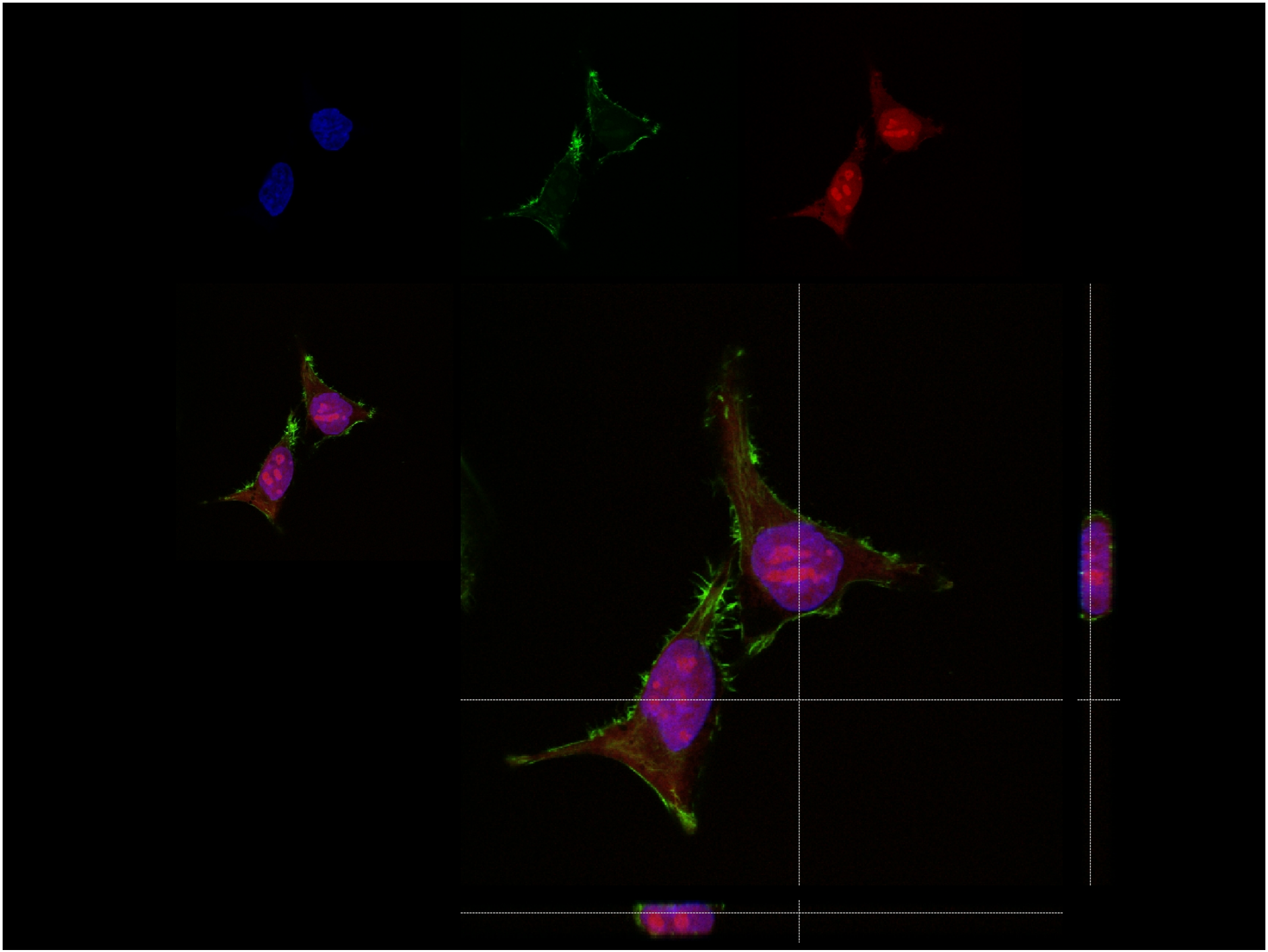
- 中研院細生所 -

XYZ projection



LEICA TCS SP5 AOBS

- 中研院細生所 -




LEICA TCS SP5 AOBS
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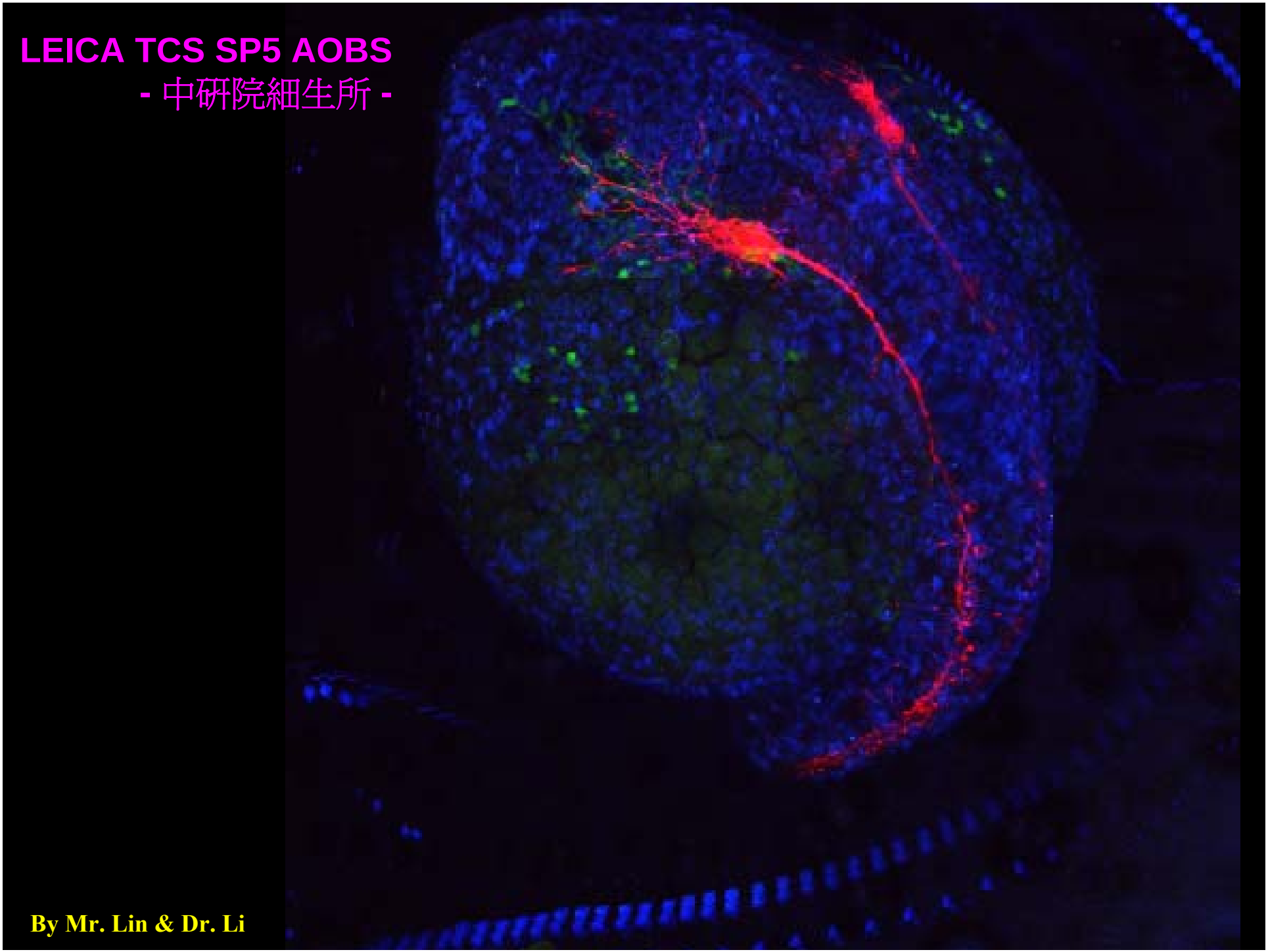
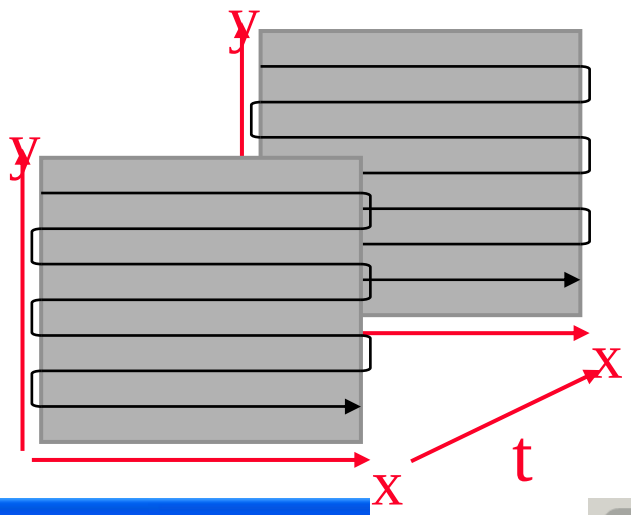
LEICA TCS SP5 AOBS

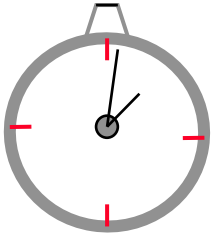
- 中研院細生所 -



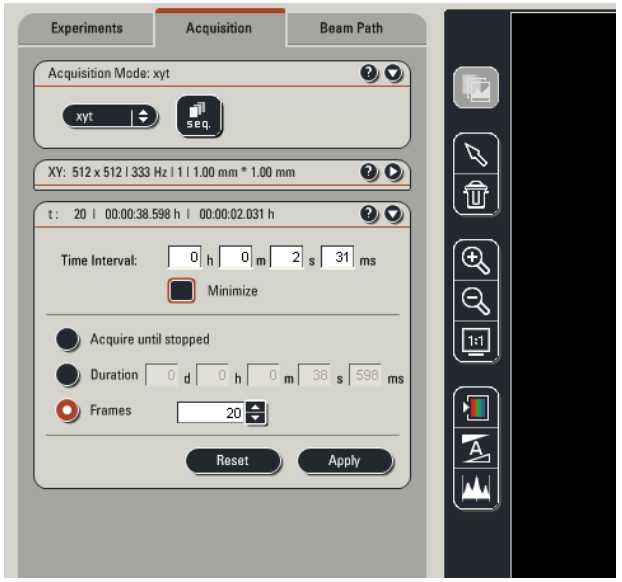
By Mr. Lin & Dr. Li

xyt





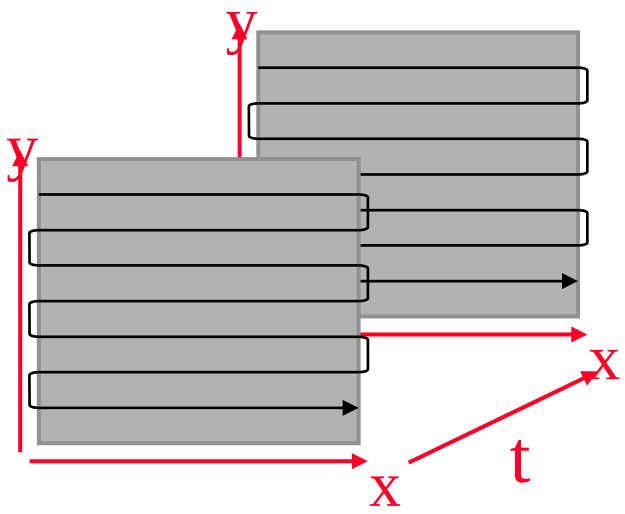
t >= xy acquisition time

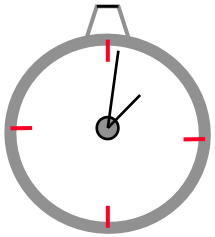


Conventional mode – High resolution

Max. resolution: 8192 x 8192
Max. speed: 5 fps at 512 x 512
25 fps at 512 x 16

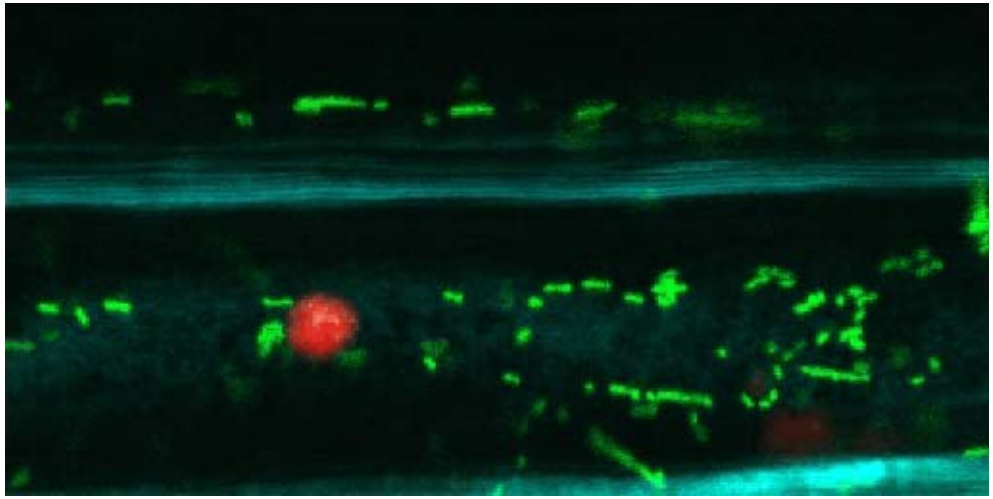
xyt





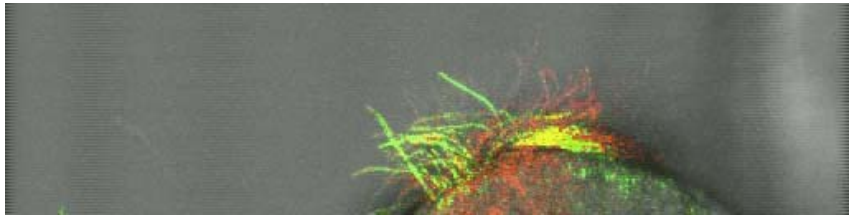
Dynamics life cell research

- Protein/organelle transport using FRAP
- Uncaging
- Physiological activity (ion measurements
- ROI based quantification



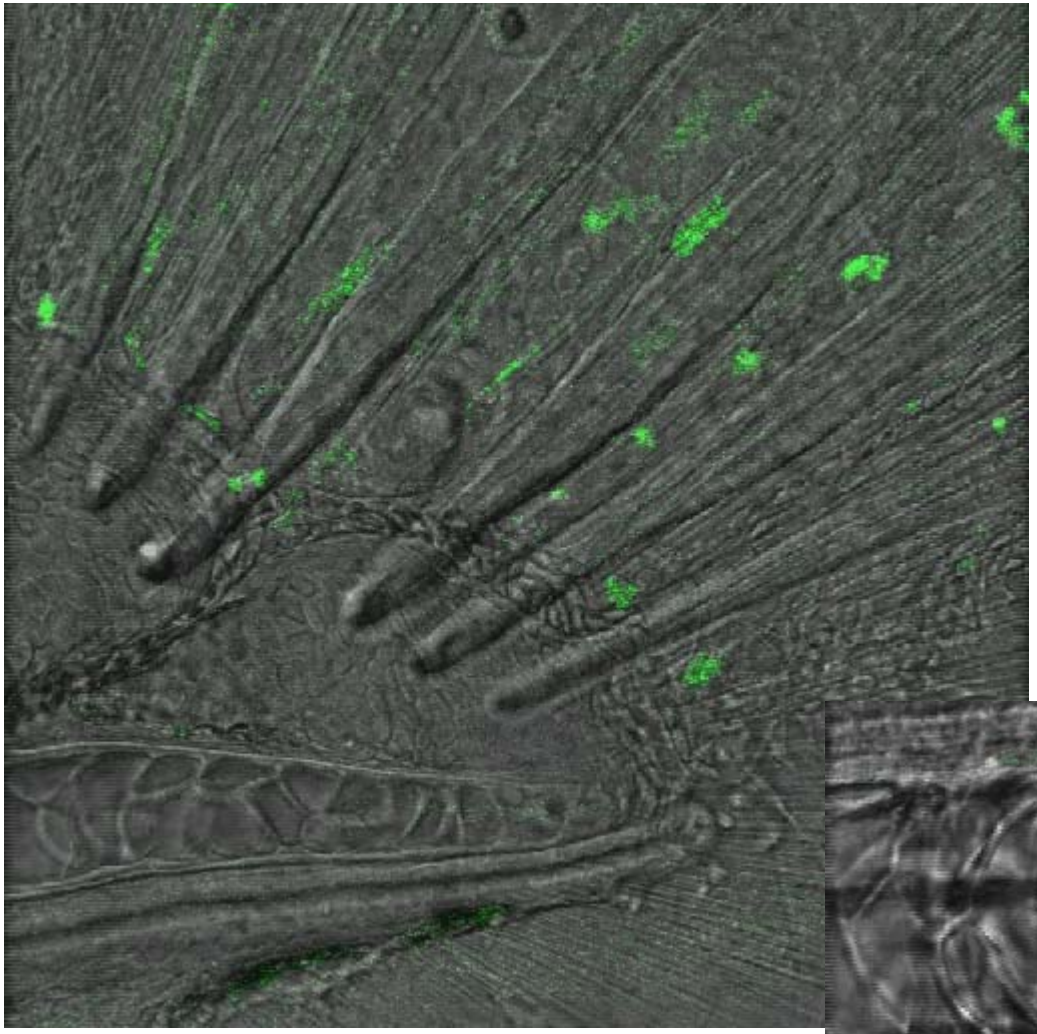
Arabidopsis thaliana
First channel: Cell wall in reflection.
2 & 3 channel: Monitoring mitochondrial (GFP-green) and plastid (autofluorescence-red) movement.

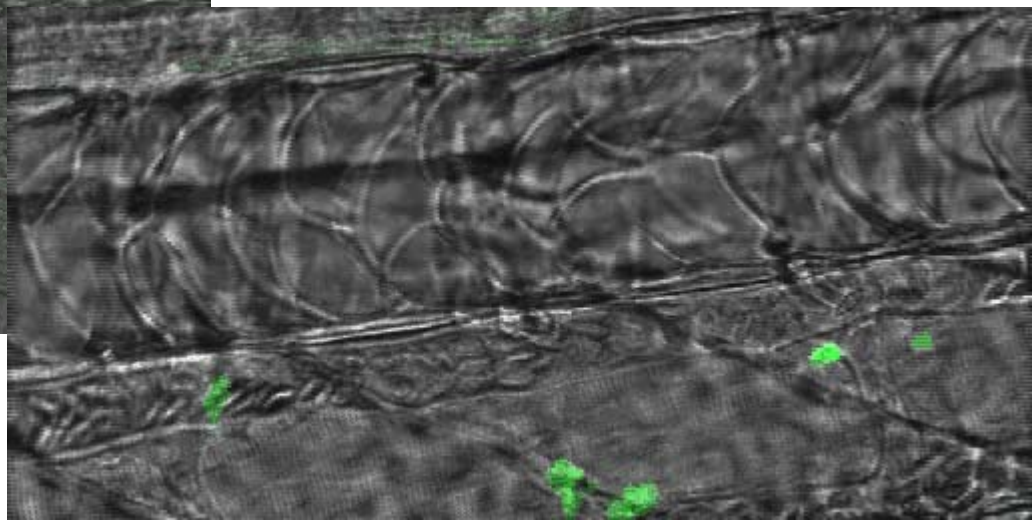
Courtesy of Prof. Dr. D. Menzel, Institut für Zelluläre und Molekulare Botanik Zellbiologie der Pflanzen, Bonn University.



Platynereis
Reflection
Fluorescence (Dil)
TLD
Courtesy of
Kristin Tessmar und Detlev Arendt (EMBL), Heidelberg, Germany

xyt





Fish embryo (Medaka)
Flow of red blood cells and migration of macrophages.
Macrophage: YFP and RFP (1st & 2nd ch)
Red blood cells: TLD

Courtesy of Clemens Grabher and Jochen Wittbrodt Clemens Grabher (EMBL), Heidelberg, Germany

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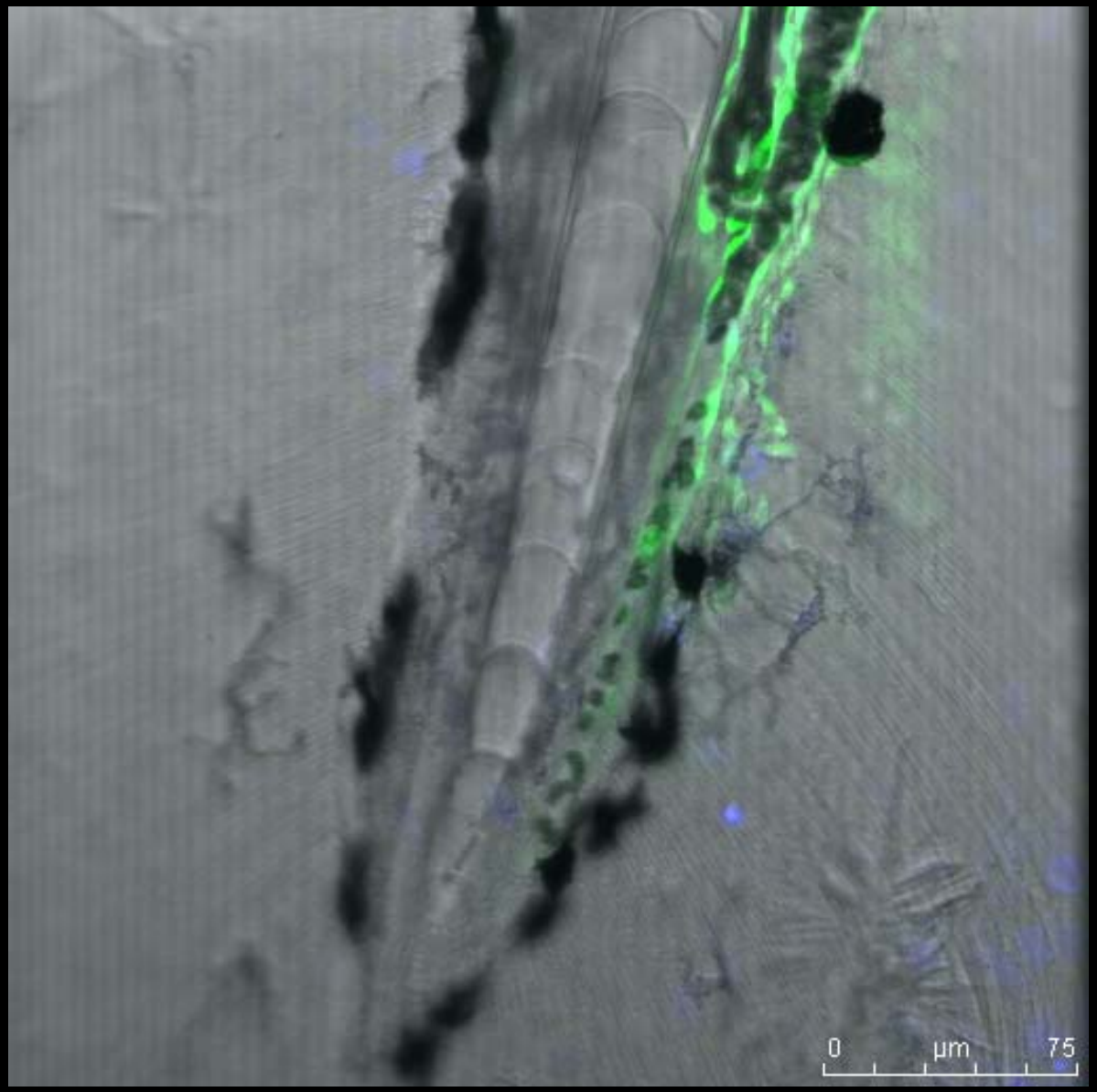
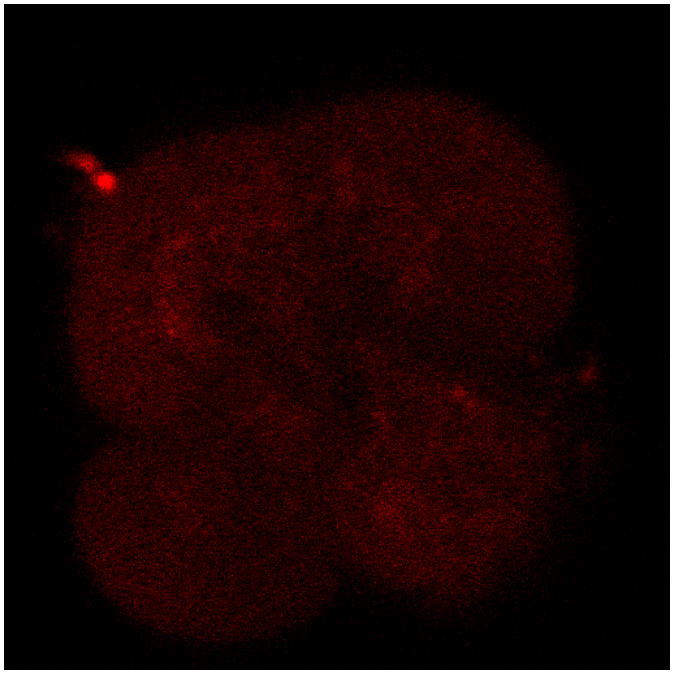


By Mr. Lin & Dr. Li

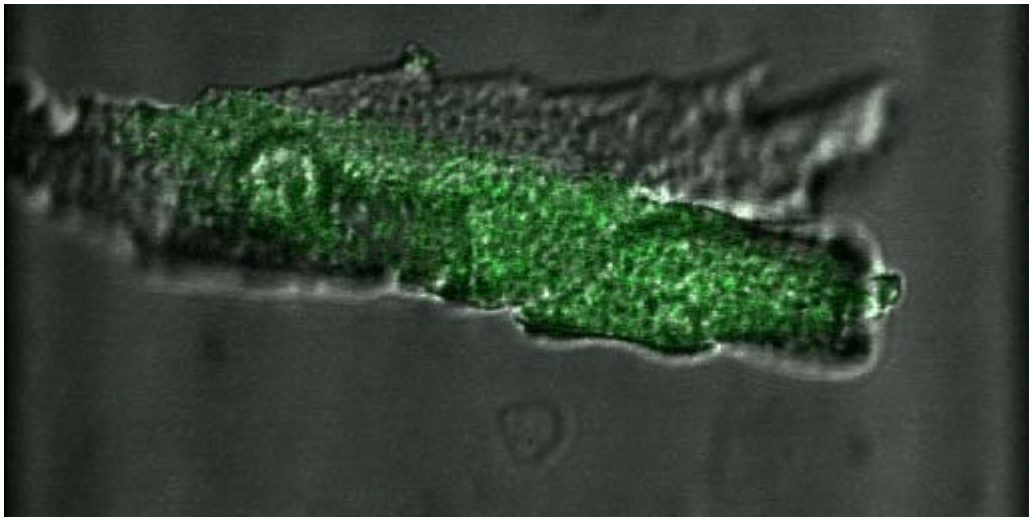
xyt - *uncage*

- Dynamic live cell imaging and kinetic measurements
- Crisp thin optical sections
- Full multiparameter fluorescence performance
- Brighter images due to lower triplet populations
- Overview and details by zoom and pan



Ca^{2+}-imaging:
UV-uncaging
Ca-Indicator: Fluo 4,
488 nm
Pancreatic acinar cells

Courtesy:
Dr. Julia Gerasimenko
Dept. of Physiology
Univ. of Liverpool

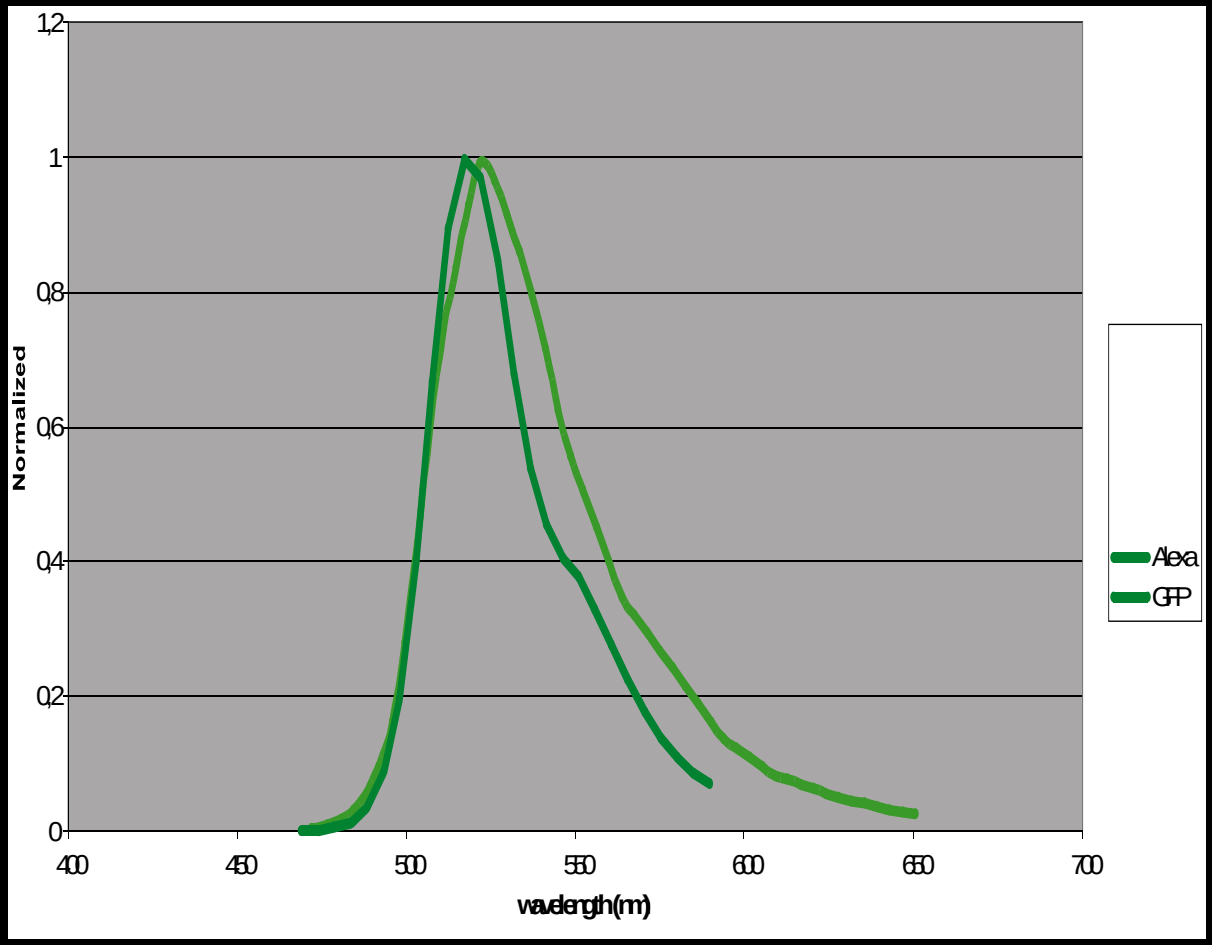


Cardiac muscle cell
Calcium waves & sparks,
1st ch Fluo 3
2nd ch TLD

Courtesy Prof. Neumann,
University Halle, Germany

xyλ Scan

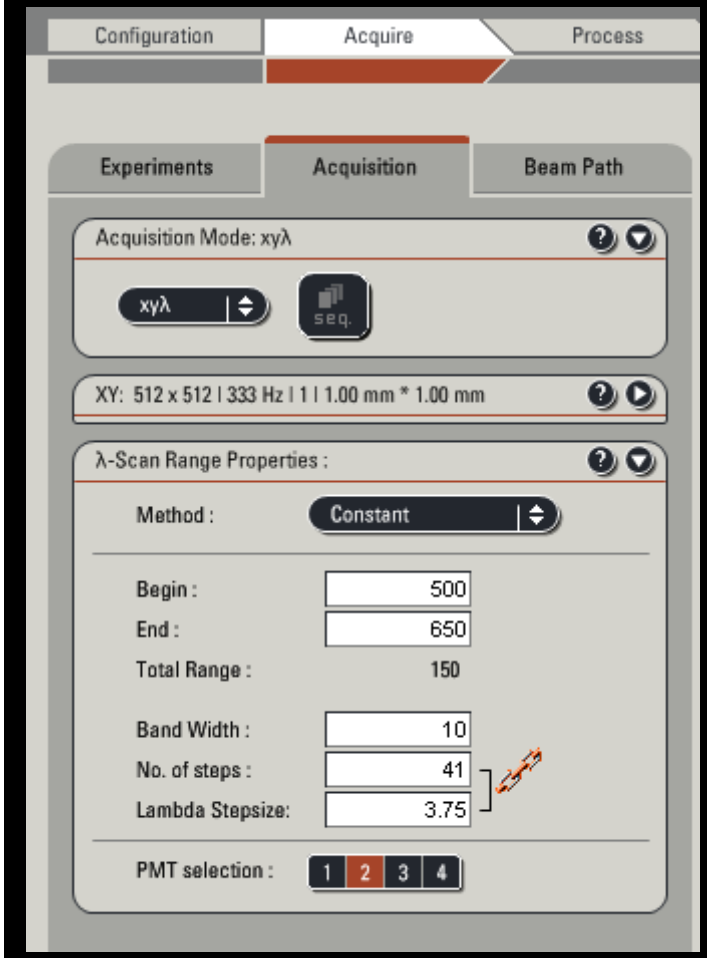
SP performance test

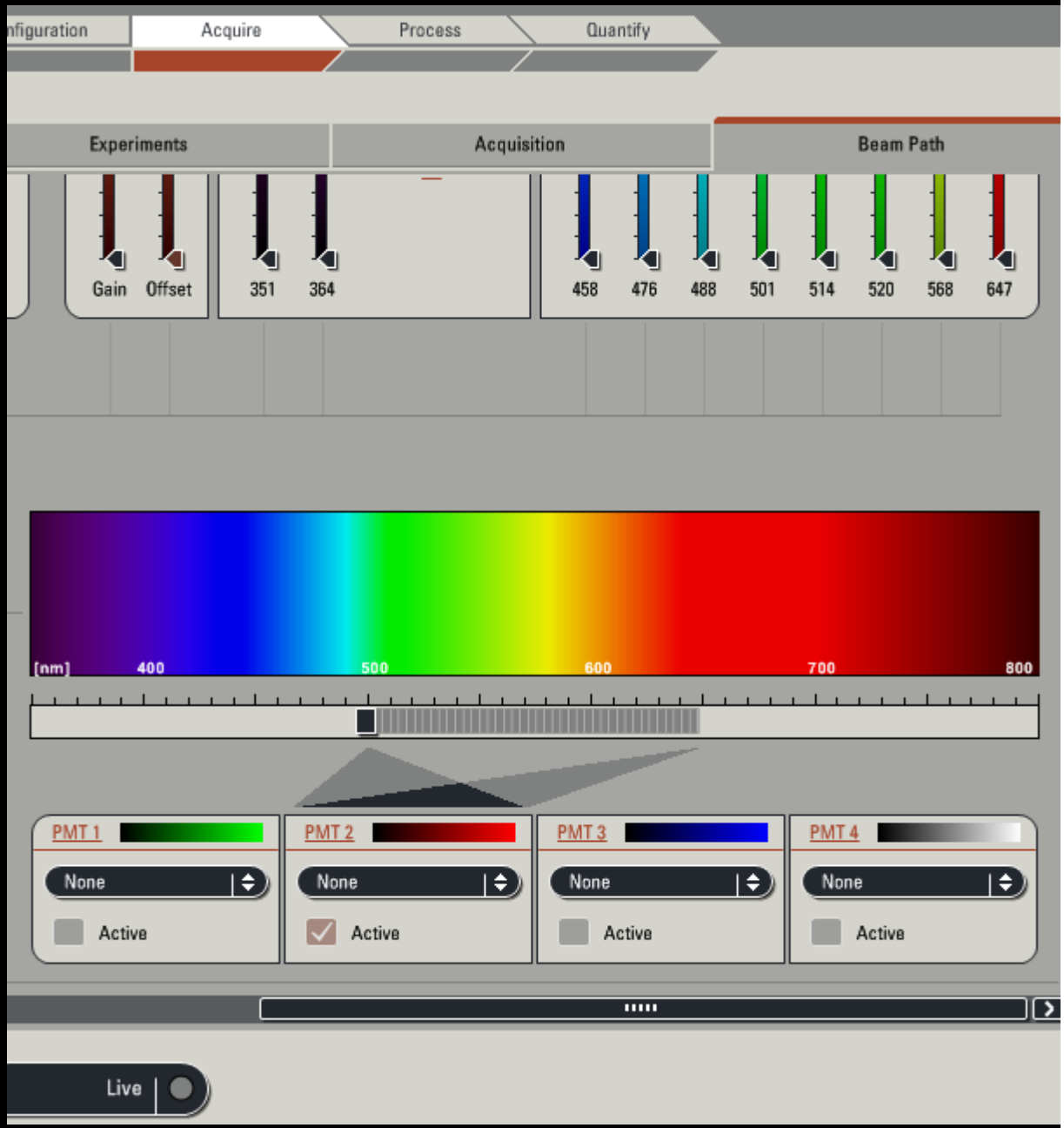


Alexa 488 – GFP

Almost complete spectral overlap

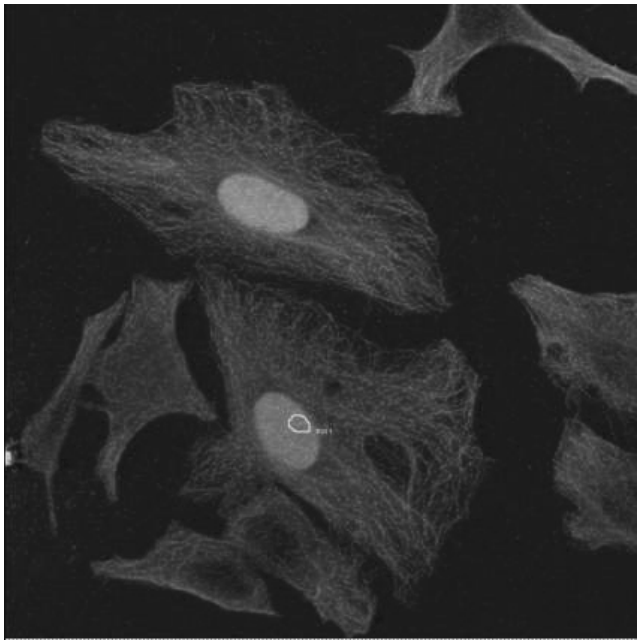
xyλ Scan


Configuration
Acquire
Process
Experiments
Acquisition
Beam Path
Acquisition Mode: xyλ
xyλ
seq.
XY: 512 x 512 | 333 Hz | 1 | 1.00 mm * 1.00 mm
λ-Scan Range Properties :
Method :
Constant
Begin :
500
End :
650
Total Range :
150
Band Width :
10
No. of steps :
41
Lambda Stepsize:
3.75
PMT selection :
1 2 3 4

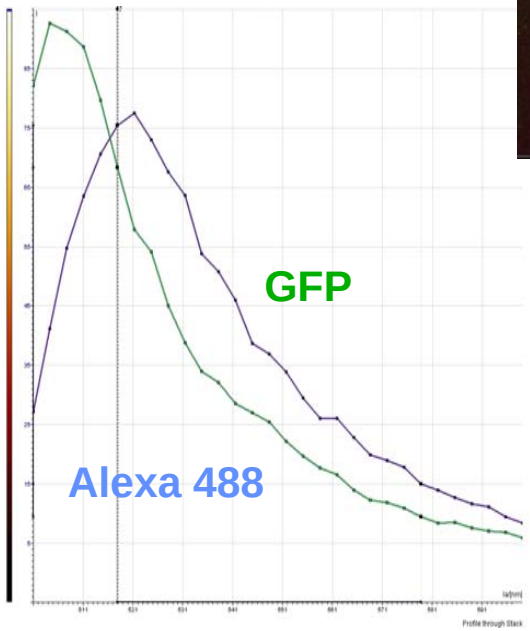

nfiguration
Acquire
Process
Quantify
Experiments
Acquisition
Beam Path
Gain
Offset
351
364
458
476
488
501
514
520
568
647
[nm]
400
500
600
700
800
PMT 1
PMT 2
PMT 3
PMT 4
None
None
None
None
Active
Active
Active
Active
Live

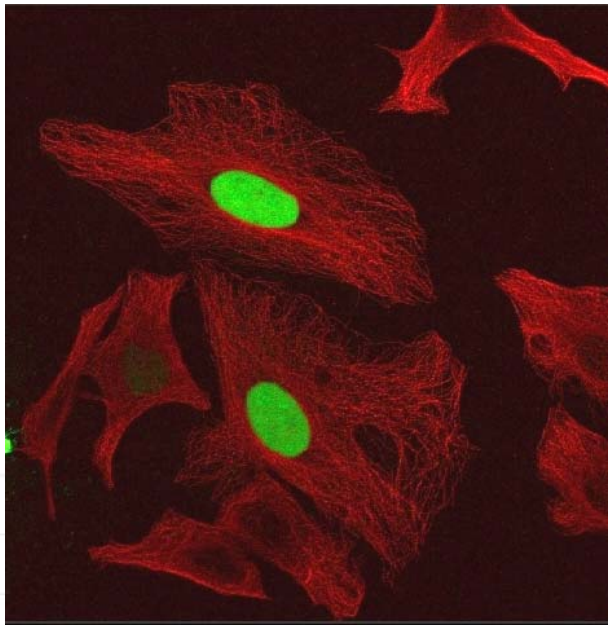
Emission control: SP performance test

Lambda scan, Leica dye finder software



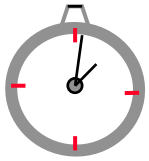
Raw image

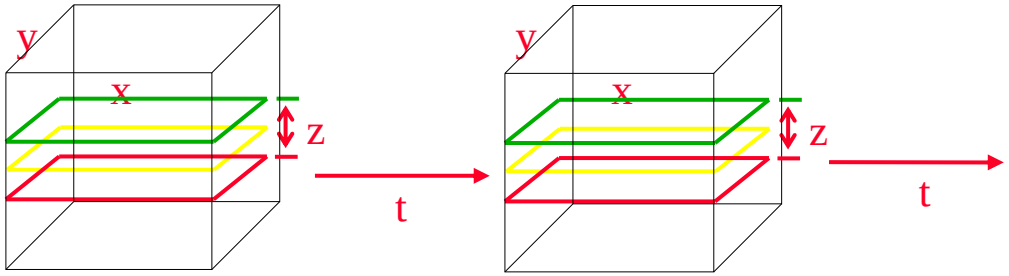


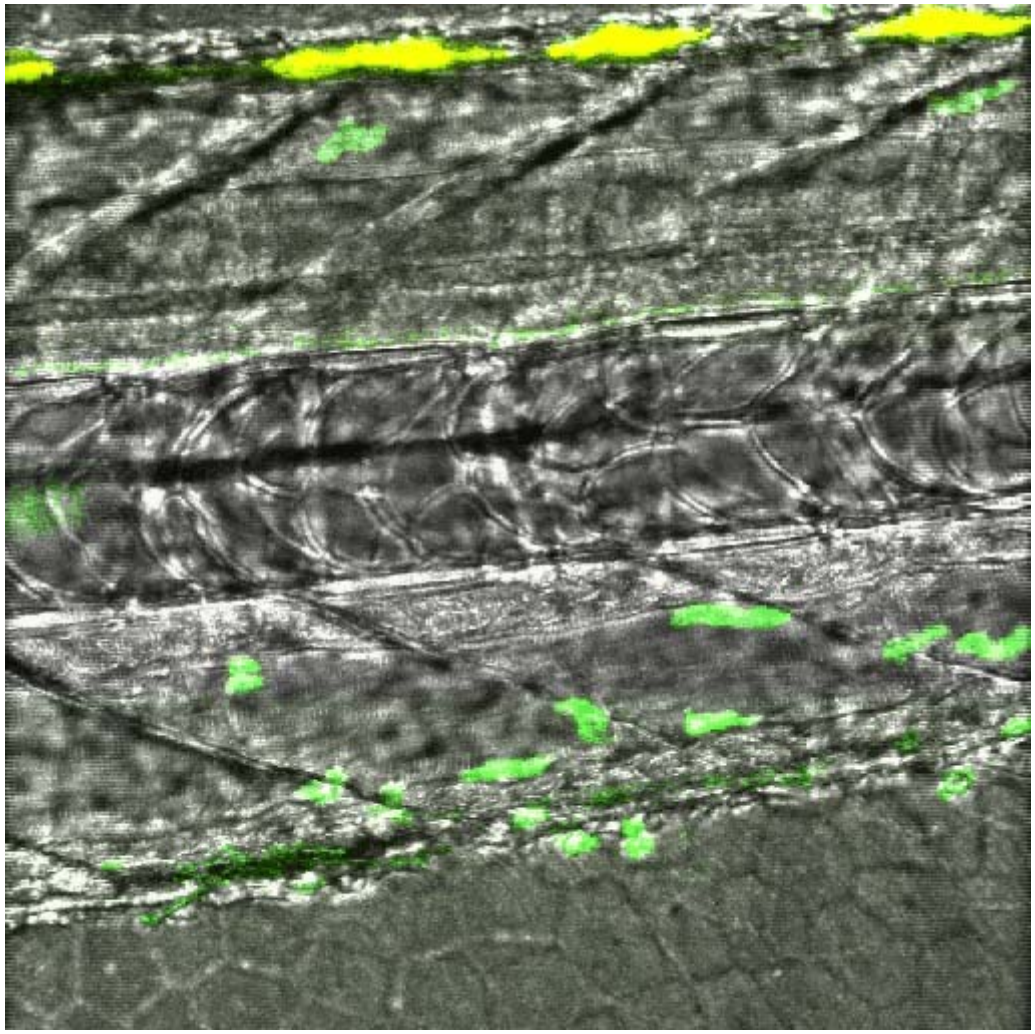


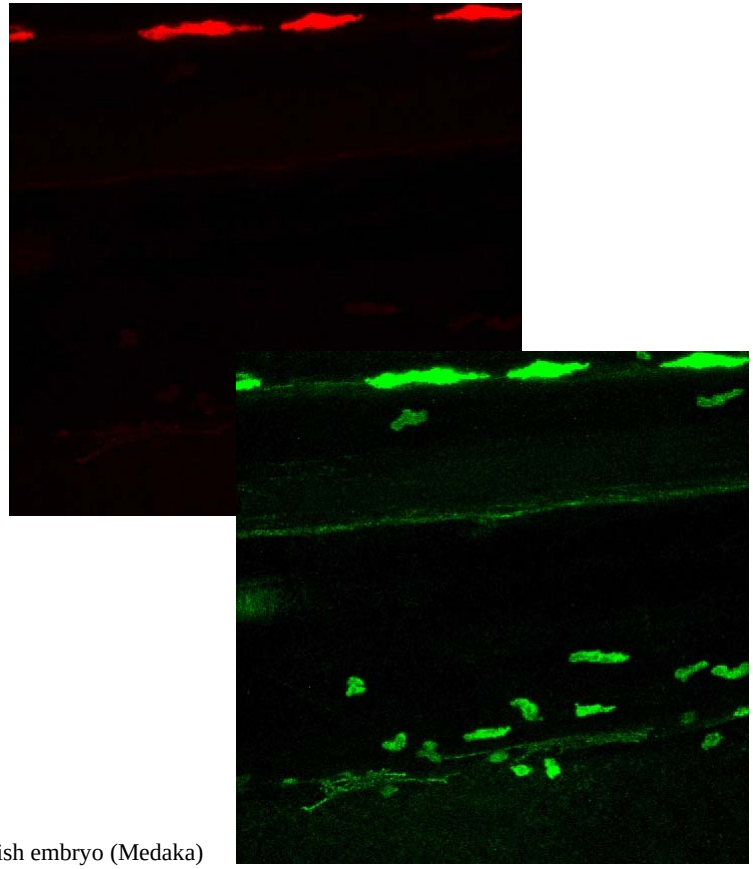
Processed with Dye Finder:
Clear dye separation

xyzt







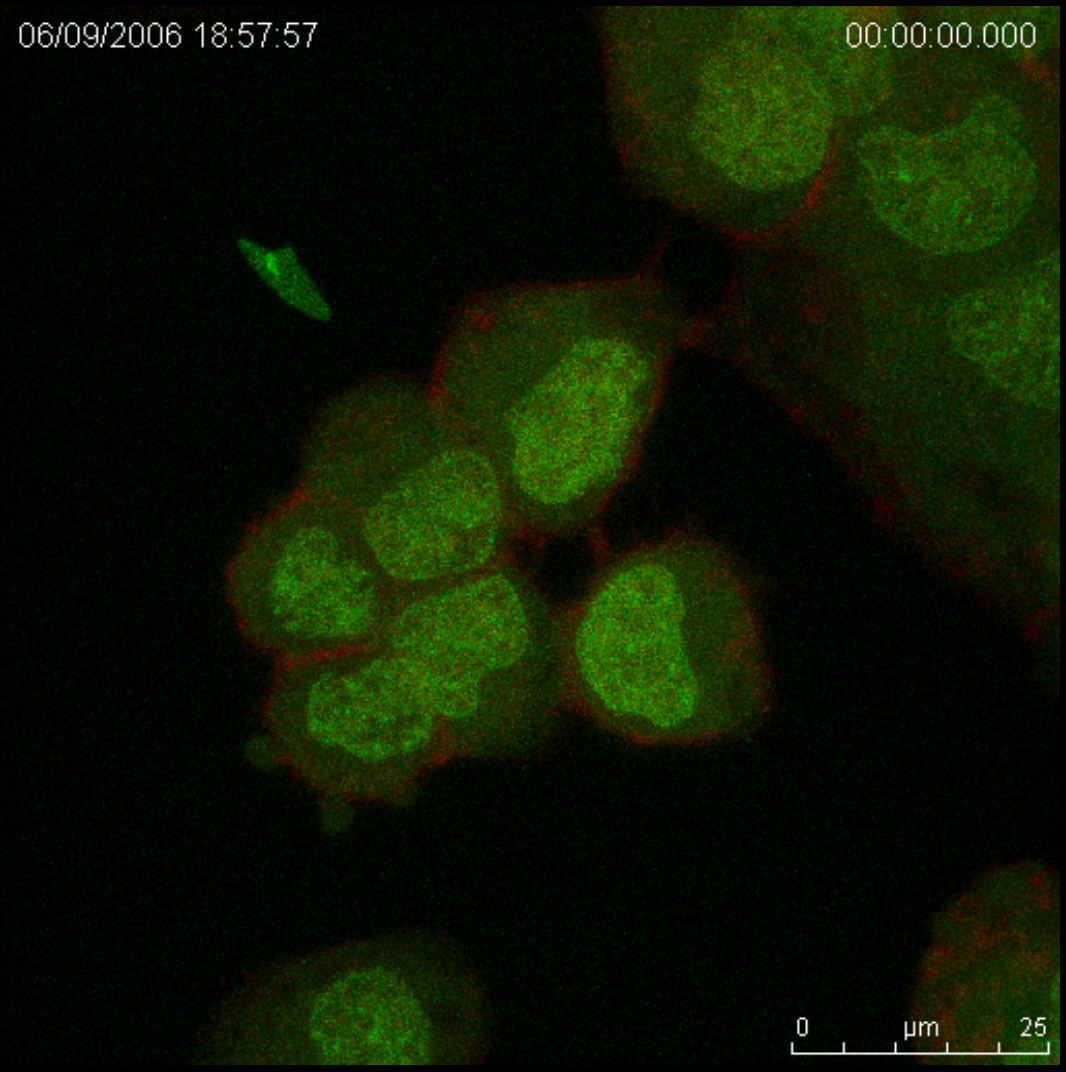


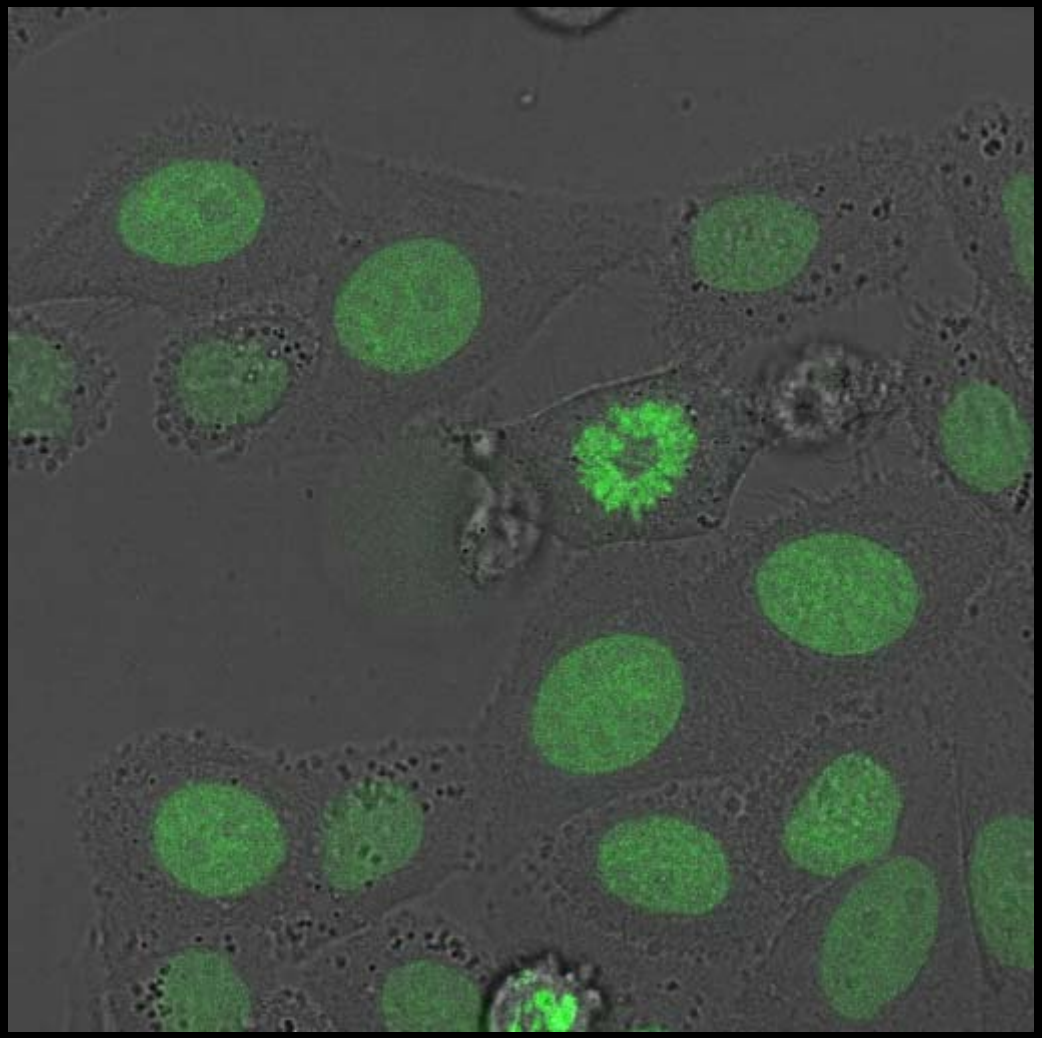
Fish embryo (Medaka)
Flow of red blood cells and migration of macrophages.
Macrophage: YFP and RFP (1st & 2nd ch)
Red blood cells: TLD

Courtesy of Clemens Grabher and Jochen Wittbrodt
(EMBL), Heidelberg, Germany

LEICA TCS SP5 AOBS

- 中研院細生所 -





Thank you!