Leica TCS SP8 X

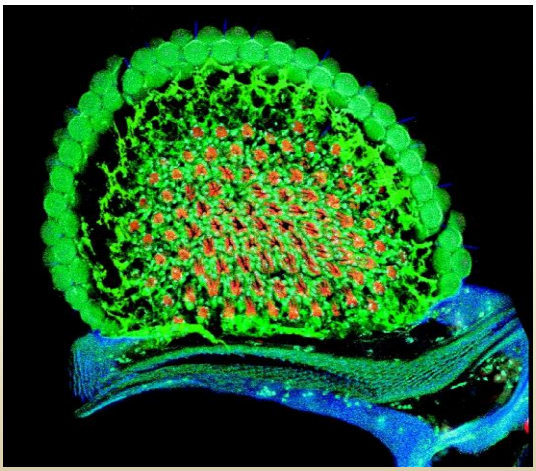
The New Milestone of Confocal Microscopy

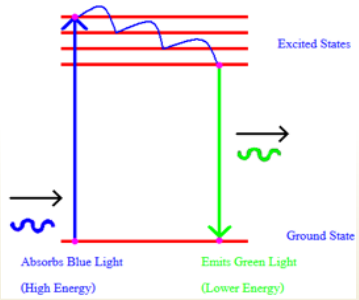
- New full range confocal platform -

Leica MICROSYSTEMS

Major Instruments Co., Ltd.

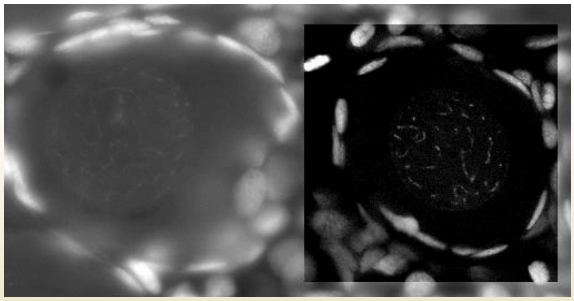
Confocal Image -- Fluorescence





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

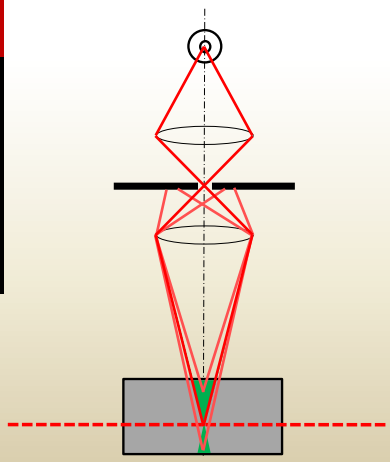
Confocal Microscopes can optically remove all information that is from outside the depth of focus.

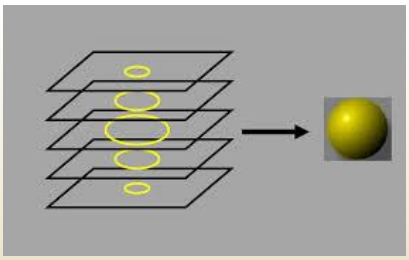


Optical Section

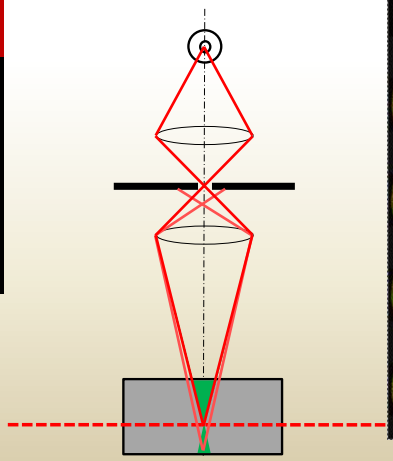
The consequence is a sharp optical section.

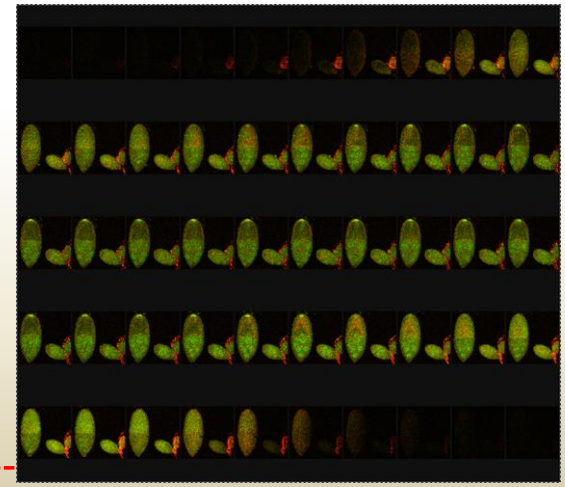
The Optical Knife:





The Optical Knife:

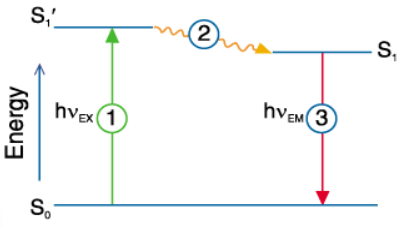




LEICA Confocal Microscopy

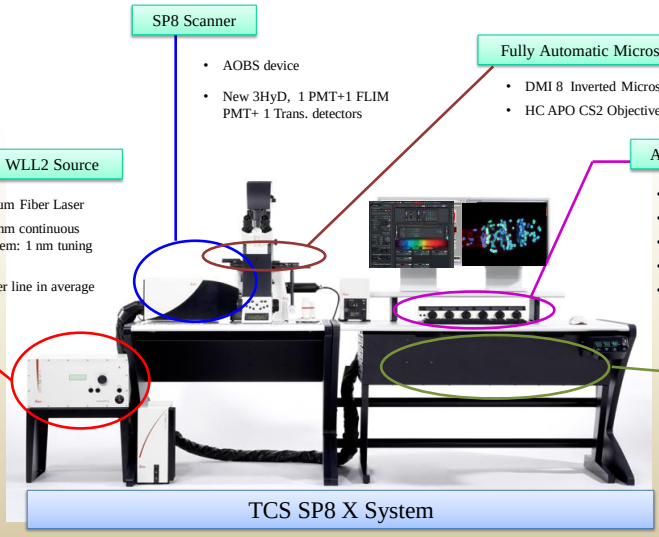
Confocal System

Main configuration :

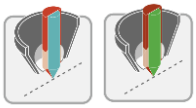


1. **Microscope System**
2. **Illumination (Excitation) System**
3. **Confocal Scanning & Detection (Emission) System**
4. **Data Control System & Application Software**
5. **Sample preparation**

LEICA TCS SP8 X AOBS Confocal Microscope



Leica DMI 8 Inverted Microscope



CS Objectives

Objective	Immersion	coverglass
HC PL APO 10x/0.4 CS	Air	0.17mm
HC PL APO 20x/0.7	Air	0.17mm
HC PL APO 40x/1.30 Oil CS2	Oil	0.17mm
HC PL APO 63x/1.40 Oil CS2	Oil	0.17mm
HC PL APO 100x/1.4 Oil CS2	Oil	0.17mm

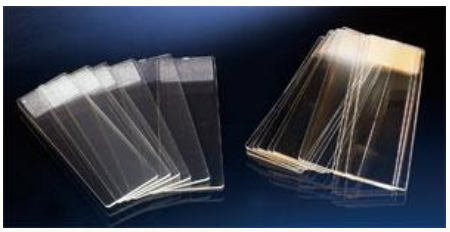
- CS: Confocal Scan, the highest quality objectives
- New set of objective lenses for UV/VIS
- Optimized for multi-color overlay between 405 nm and VIS lasers
- Excellent planarity of field

Sample preparation

✓ Fixed sample

Slide + #1.5 coverglass

Inverted



✓ Chamber

I. Multiwells devices

Not recommend for confocal microscopy!

II. Plastic Petri dishes

Only LWD objectives:
HC PL FLUOTAR 10x/0.30 PH1
HCX PL FLUOTAR L 20x/0.40 CORR PH1
HCX PL FLUOTAR L 40x/0.60 CORR PH2

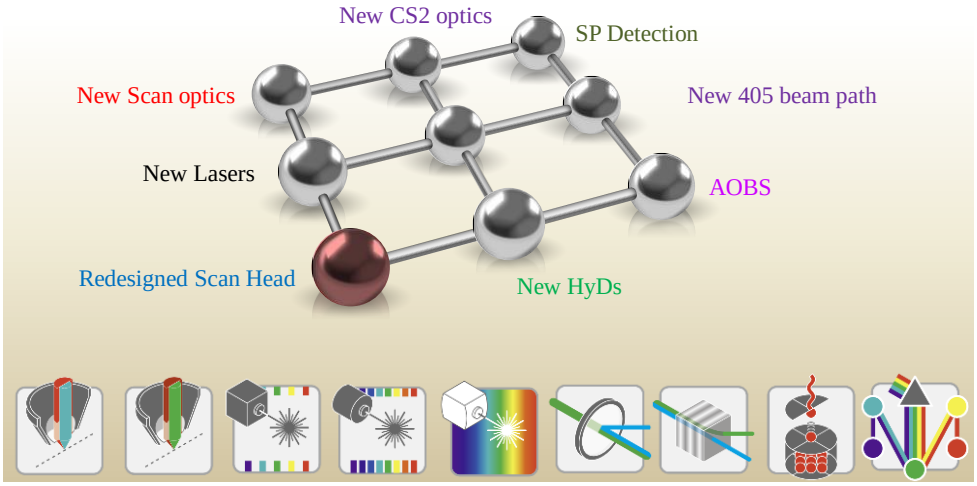
LEICA TCS SP8 X AOBS Confocal Microscope

Leica TCS SP8

Looking forward to your discoveries

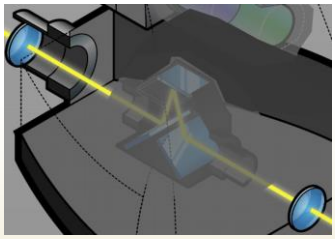
- Sensitivity by Design
- Faster for new Biology
- New technology for image
- Ready to grow

Leica TCS SP8
Sensitivity by design

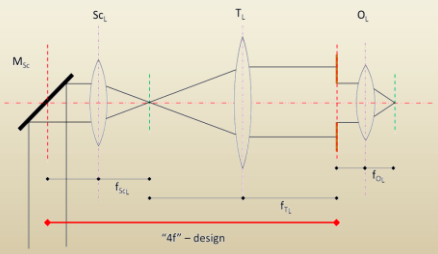


New scan optics

- New coatings for improved transmission
- Dedicated scan optics maximizing photon efficiency
  - optimal transmission for each application
- Arranged in “4f” design for even field illumination
- “My Confocal” – dedicated intermediate optics



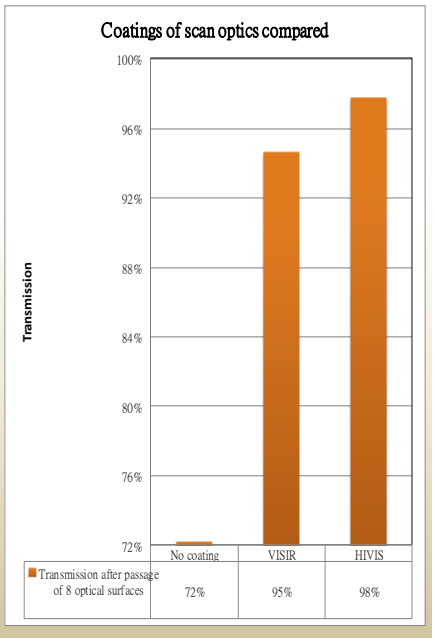
Always choose the right scan optics for your system

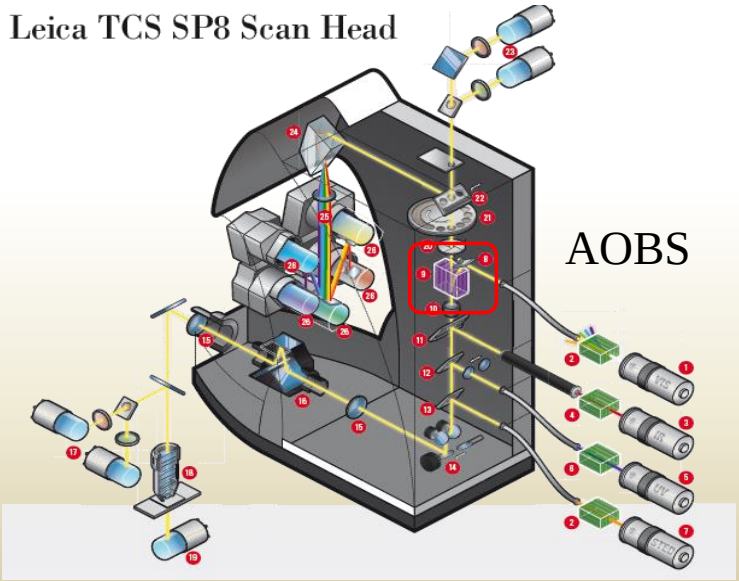


New scan optics

	T[%] per opt. surface	T[%] after passage of 8 opt. surfaces
No coating	96%	$(96\%)^8 = 72\%$
VISIR (400-1300nm)	99.3%	$(99.3\%)^8 = 95\%$

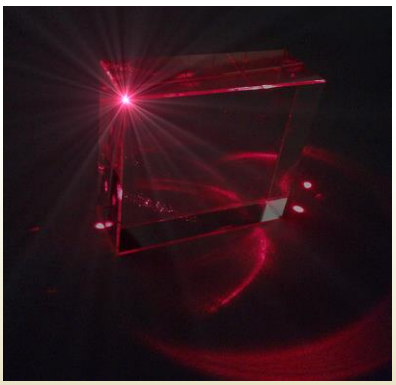
Reduced reflection by 60 %



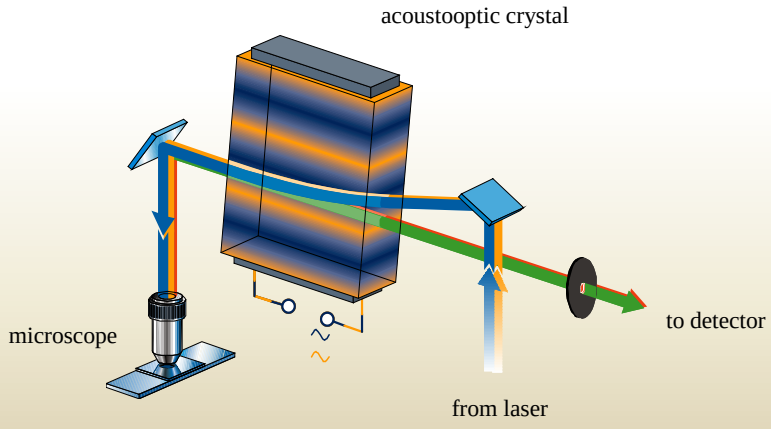


AOBS

- **Highly transparent** → Increase sample lifetime, cell viability → more and better experiments
- **Flexible** → links in with WLL, Ar laser → more diverse experiments
- **Ease of use** → Save training time; better turnover (low failure rate)
- **Supports new dyes** → Future-proof
- Cool technology → Prestige



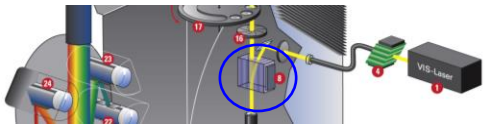
AOBS Principle



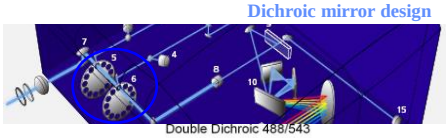
....up to 8 lines simultaneously!

AOBS vs. Dicroic mirror

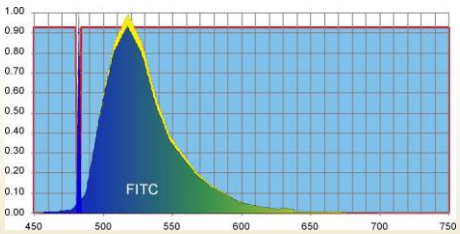
LEICA TCS SP8 AOBS

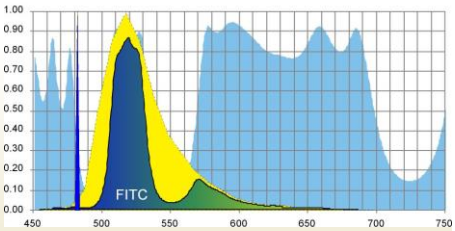


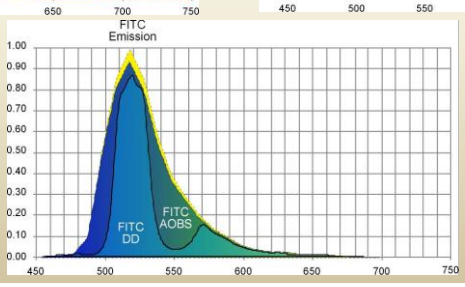
Dichroic mirror design



Double Dichroic 488/543
(measured curve)

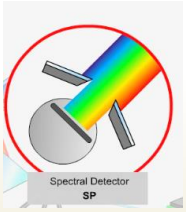


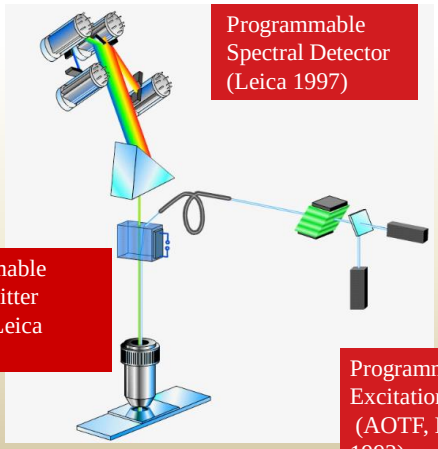




Confocal microscopy: sophisticated

Leica Innovation Goals: Introduction of tuneable optical elements





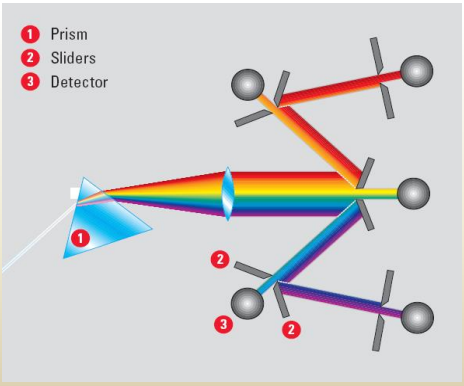
Programmable Spectral Detector (Leica 1997)

Programmable Beam Splitter (AOBS, Leica 2002)

Programmable Excitation Filter (AOTF, Leica 1993)

Leading in Multispectral Imaging:

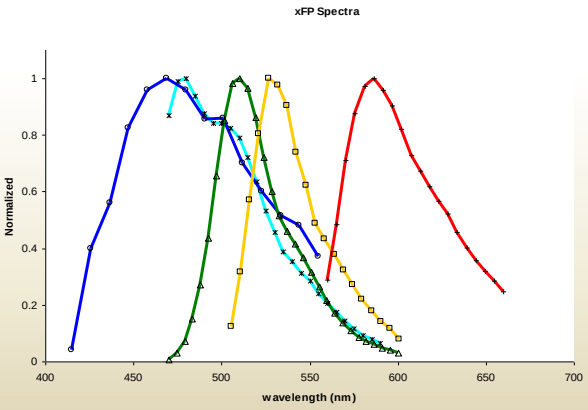
Spectral Imaging Detector SP



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

Spectral scans

No distortions in spectra caused by filter or splitter transmission spectra

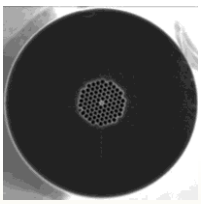


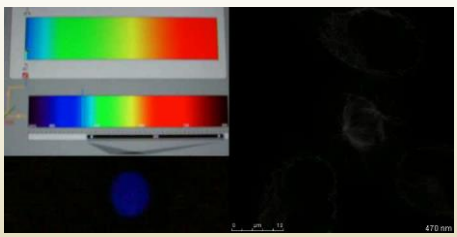
Courtesy: T. Zimmermann, EMBL Heidelberg

BFP exc: 405nm
CFP exc: 458nm
eGFP exc: 476nm
YFP exc: 488nm
dsRed exc: 543nm

New laser system

- Laser Kit **White light laser**
- 470-670nm
- WLL now supports
  - Lambda² Excitation-Emission scans
  - Lightgate (reflection removal)
- Add optional UV lasers
- LightGate: Non-optical reflection removal
- Pulsepicker option for improved FLIM
- Lifetime Saver





White light laser

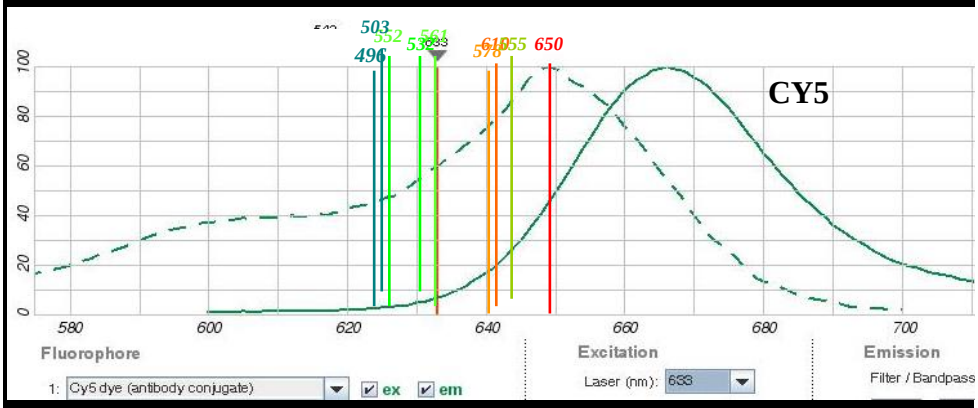
共軛焦顯微鏡中白光雷射的應用

- 雷射要Tune出連續波長並不困難！e.g. MP(IR) laser 680-1080nm
- 重點在於共軛焦系統能否接收雷射所tune出的任意波段？
  AOBS – Yes!, Dichroic – No!
- 重點在於共軛焦系統能否接收雷射所tune出的任意波段的任意組合？
  AOBS – Yes!, Dichroic – Impossible!
- 重點在於能否一次同時導入8個任意波段的激發光雷射？
  AOBS – Yes!, Dichroic – Impossible!

✓ 只有搭配在AOBS系統上，才能真正稱之為白光雷射系統。

Excitation wavelength

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



- Continuous Tunability
- Optimal excitation
- Cross-excitation adjustable

Leica HyD

- Large dynamic range
- Improved cell viability
- High-speed imaging
- Single photon counting
- Open upgrade path

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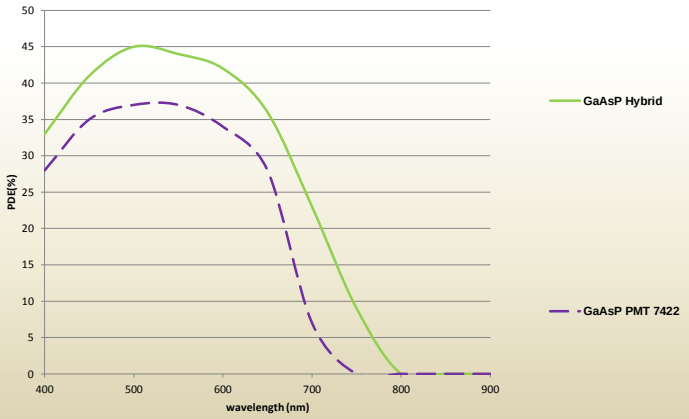
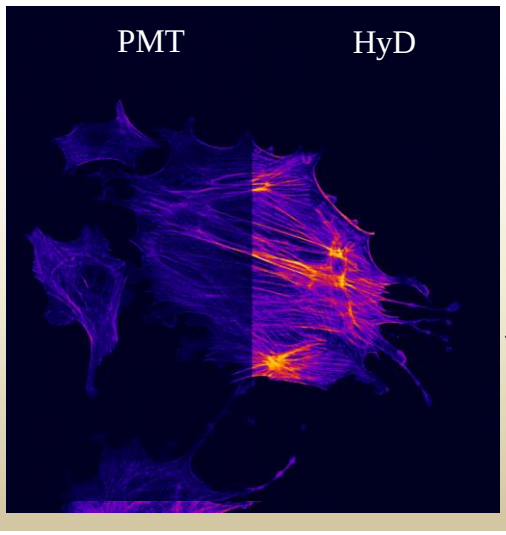
Leica HyD

High quantum efficiency



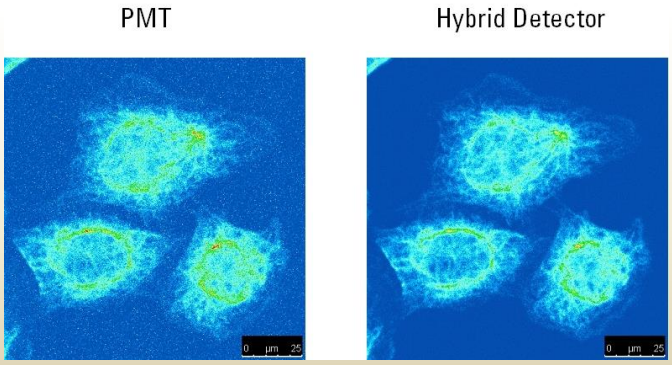
Reduced light dosage: Increased cell viability



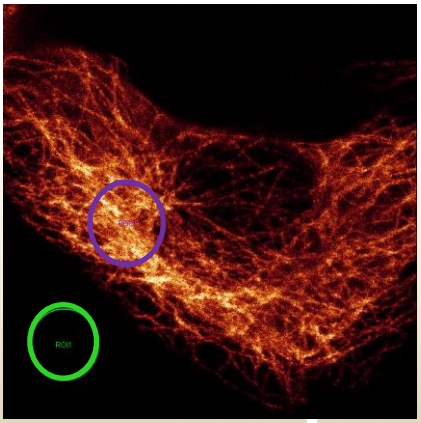
Ex 488 @ 1%
Em 490-556 nm
scan freq. 400 Hz
No accumulation
No averaging

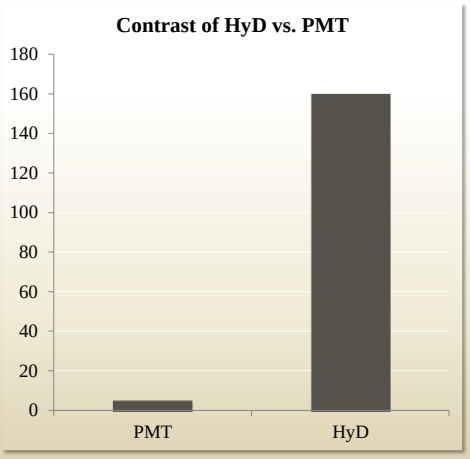
HyD

Improved Signal-to-Noise

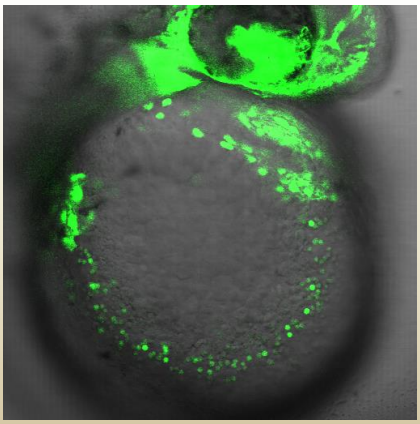


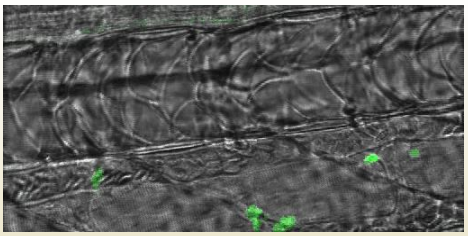
Improved contrast of HyD vs. PMT

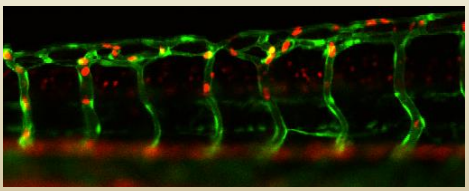




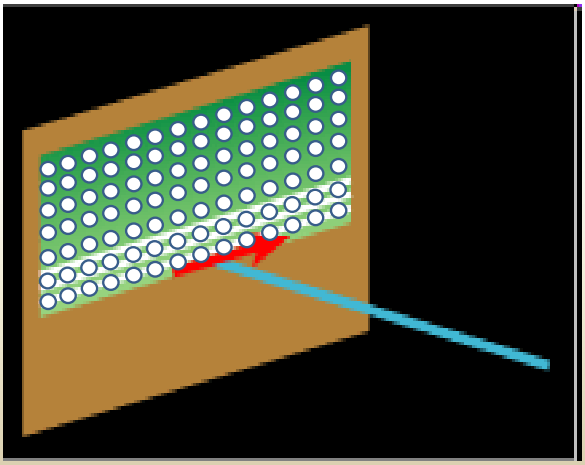
Faster for new biology





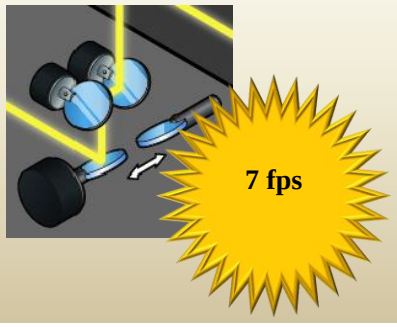


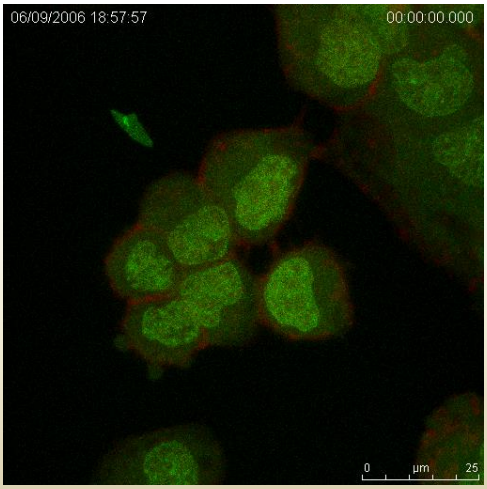
Single Point Scan Scanner



New FOV Scanner

- Better sampling at low magnification
- Large FOV
  - **Field number = 22**
  - New Zoom range 0.75x .. 48x
- Save time on post-processing (mosaics)
- Homogenous illumination -> better data
- Now with 3600 Hz scan frequency (bidir)
  - Equiv. to 7 fps @ 512 x 512





New technology for image

- λ2 -Mapping

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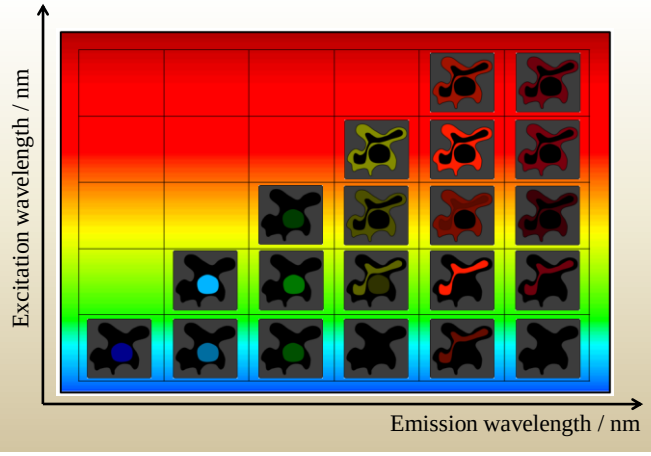
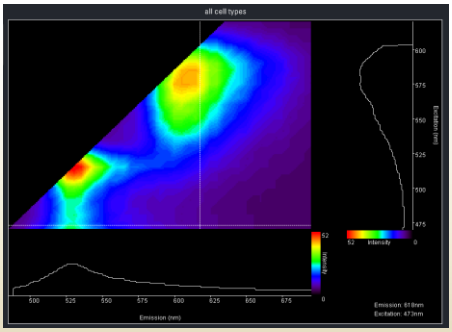
Excitation emission (λ^2) scan: What is it?

- **λ^2– scan:** new scan mode, that combines excitation Λ and emission λ scan
- **λ^2 – map:** result of λ^2– scan: a 2 dimensional fluorescence spectrum, plots fluorescence intensity over excitation and emission



λ^2 –Mapping: Discover hidden information



Benefits of λ^2 -Maps

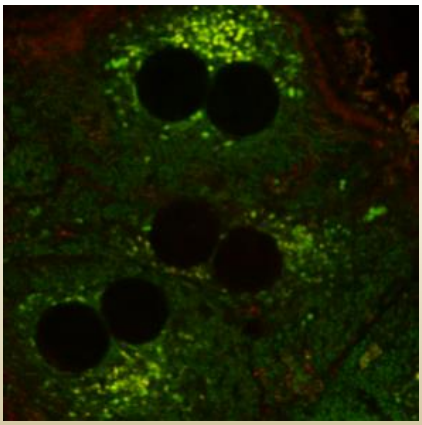
- Full spectral analysis of images
- Understand samples with very complex fluorescence
- Depict multifaceted features at one look
- Complete spectrum in each pixel
- Extract excitation and emission spectra

ADVANCED / USER CASE / APPLICATIONS

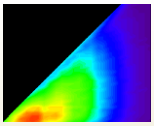
APPLICATION: FIND THE BEST FLUORESCENT LABEL

λ^2 –Mapping:
Find the best fluorescent label!

Overview image



λ^2 plots:
Excitation: 470 to 670 nm,
Detection: 500 to 750 nm



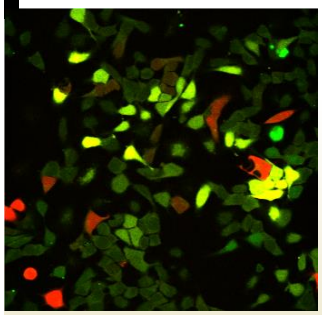
Strong autofluorescence of primary mouse hepatocytes

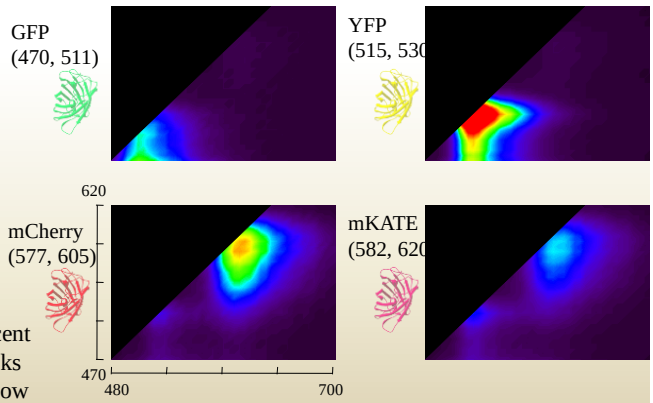
Sample: Courtesy of René Meyer, Klingmüller Group, Systems Biology of Signal Transduction, DKFZ, Heidelberg, Germany

ADVANCED / USER CASE / APPLICATIONS

APPLICATION: OPTIMIZATION OF DATA QUALITY

λ² –Mapping:
Experiment optimization for best data quality

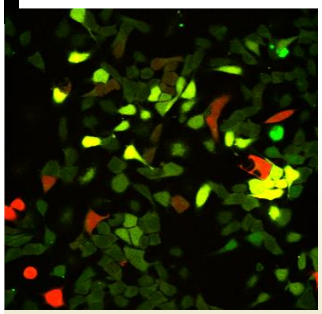


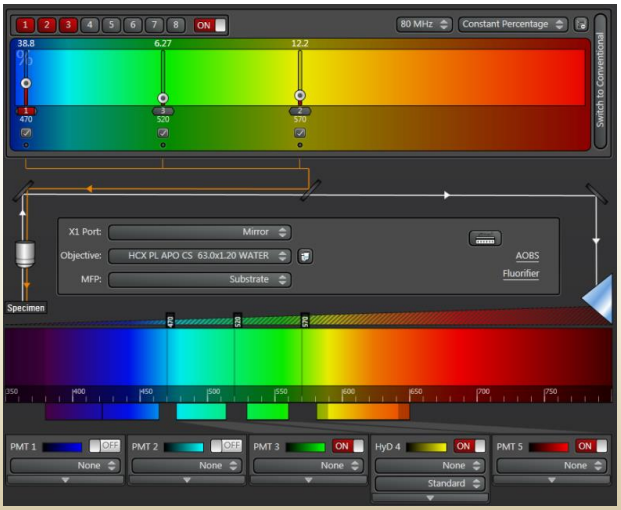


Sample: Mixture of fixed cells expressing four different fluorescent proteins. Excitation emission peaks are given in brackets. All cells show a small autofluorescence peak at (512, 533) nm.

Courtesy of Kees Jalink, Department of Cell biology, The Netherlands Cancer Institute Amsterdam, The Netherlands

λ² –Mapping:
Experiment Optimization for Best Data Quality





Sample: Mixture of fixed cells expressing four different fluorescent proteins.

Courtesy of Kees Jalink, Department of Cell biology, The Netherlands Cancer Institute Amsterdam, The Netherlands

New technology for image

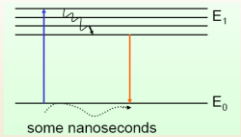
- Light Gate imaging

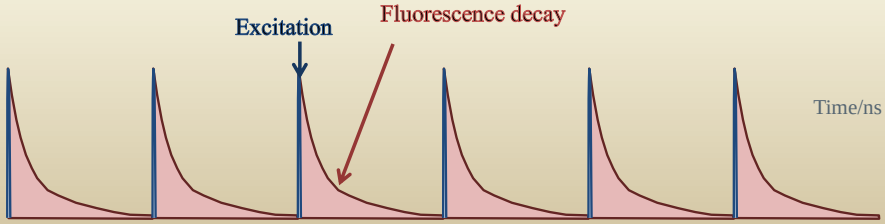
Major Instruments Co., Ltd.

TCS SP8X LightGate
Filter free removal of non-wanted background

- The WLL is a pulsed laser
- Use time information to
  ⇒ separate fluorescence decay from reflection
  ⇒ discriminate between wanted and non-wanted fluorescence

Fluorescence lifetime **Average time that molecules stay in their excited state**

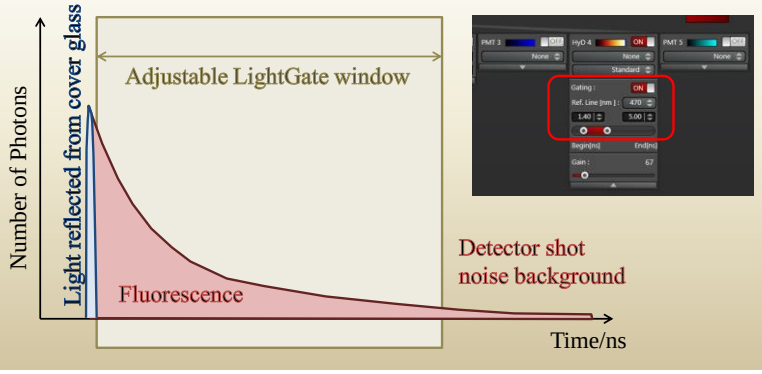




TCS SP8X LightGate
Filter free removal of non-wanted background

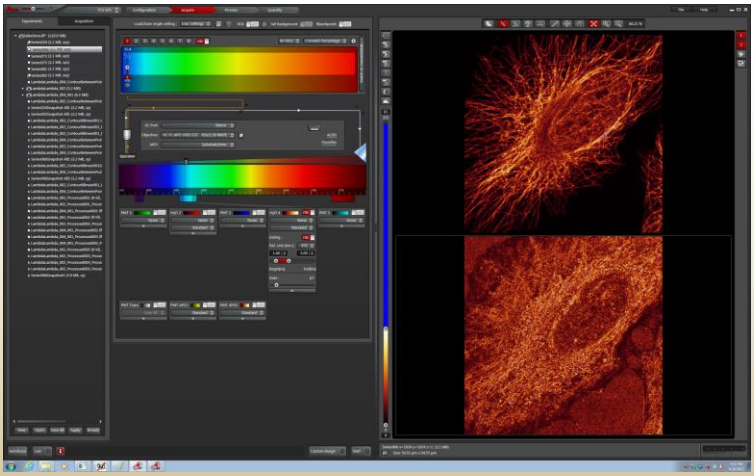
- Detector data reading is switched off during WLL pulse.
  ⇒ Only fluorescence signal is used for image formation, reflected light is excluded.

- LightGate is adjustable to different fluorescence decay times.
  ⇒ Removal of non-wanted fluorescence is light possible.



Removal of reflected and scattered light by LightGate

Sequential scan with and without LightGate.
Detection directly at excitation wavelength.



LightGate on

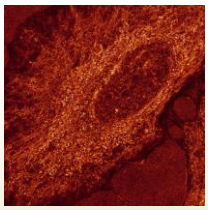
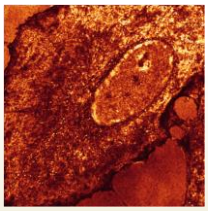
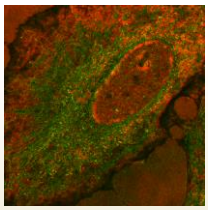
LightGate off

Removal of reflected and scattered light by LightGate

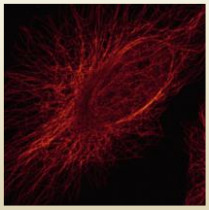
Excitation: 470 nm
Detection: 465-590nm

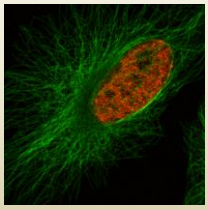
Excitation: 510 nm
Detection: 495-540 nm

Overlay

LightGate off





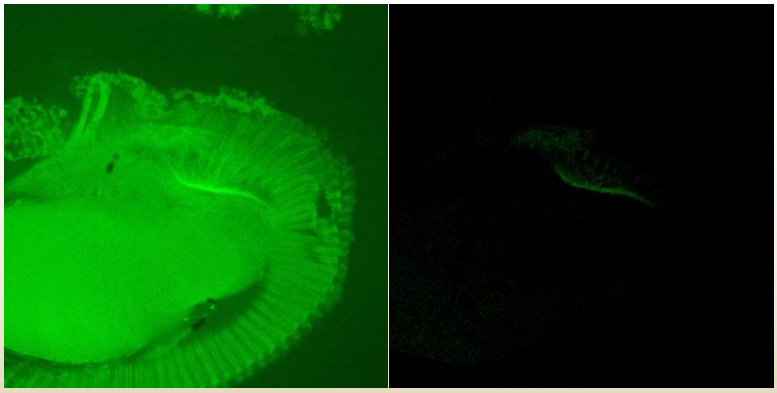
LightGate on

Sample: fixed HeLa cells, tubulin stained with BD Horizon V-500, nucleus stained with Chromeo 505, xy scan

Emission scan with Light gate on

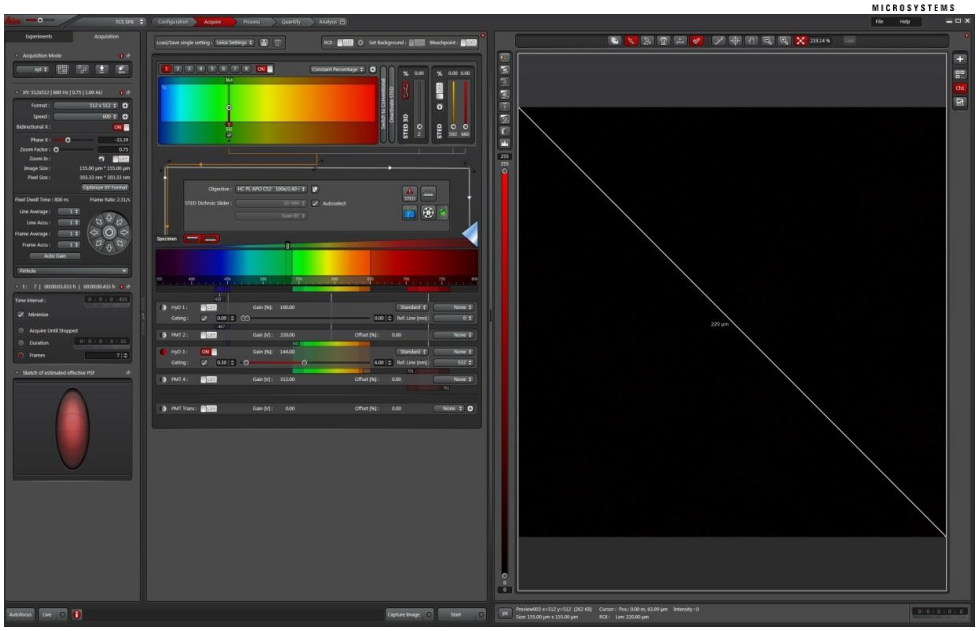
Light Gate Off

Light Gate On

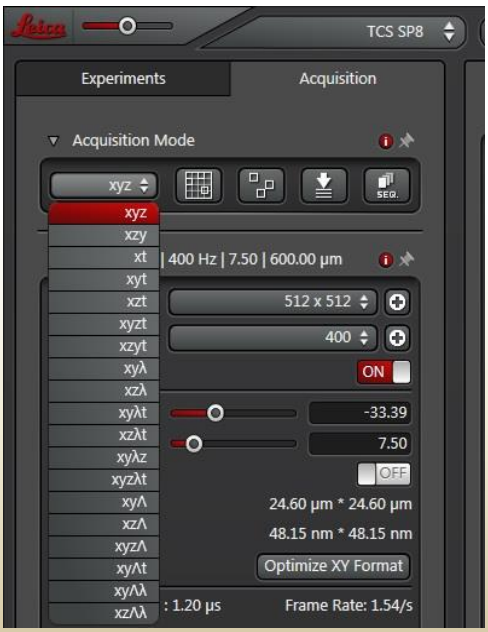


New Software
LAS X

LAS X

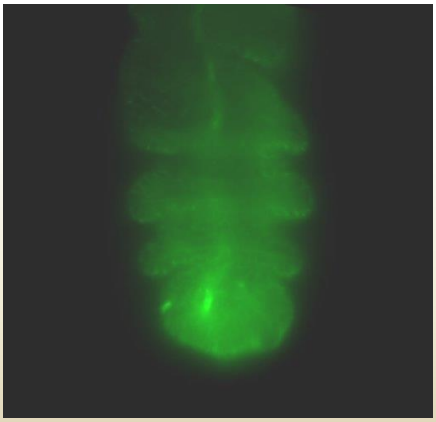


LAS X

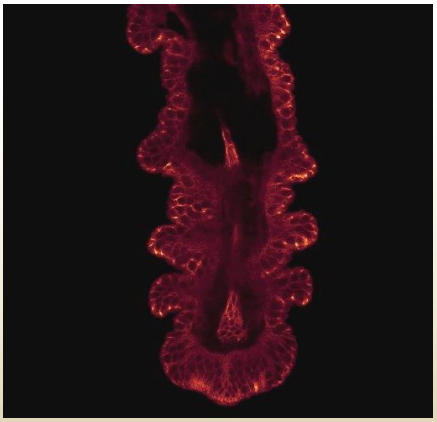

TCS SP8
Experiments
Acquisition
Acquisition Mode
xyz
SEQ.
xyz
xzy
xt
xyt
xzt
xyzt
xzyt
xyλ
xzλ
xyλt
xzλt
xyλz
xyzλt
xyΛ
xzΛ
xyzΛ
xyΛt
xyλλ
xzΛλ
400 Hz | 7.50 | 600.00 µm
512 x 512
400
ON
-33.39
7.50
OFF
24.60 µm * 24.60 µm
48.15 nm * 48.15 nm
Optimize XY Format
: 1.20 µs
Frame Rate: 1.54/s

Multidimensional
Confocal Imaging

xy

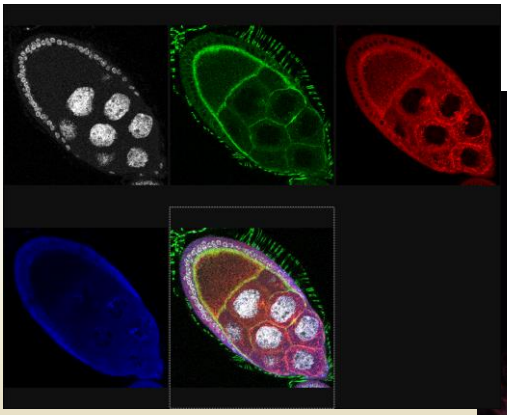


Drosophila leg, FITC, non-confocal



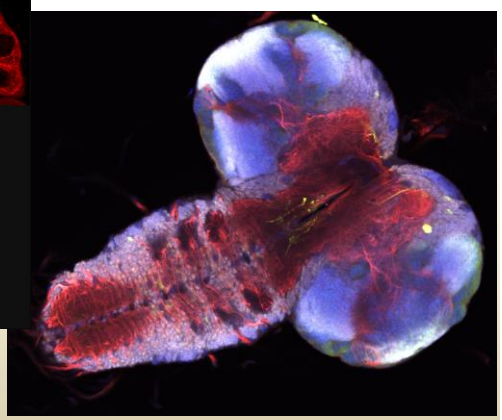
confocal

xy



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

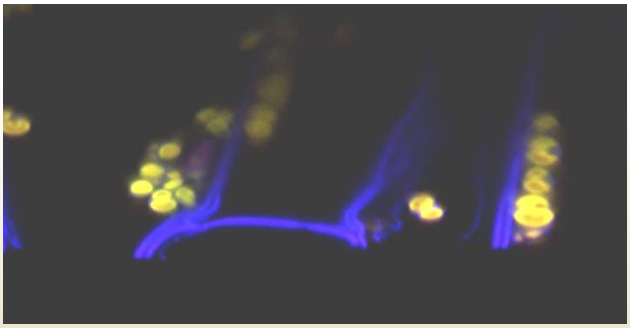
Drosophila, 4k x 4k

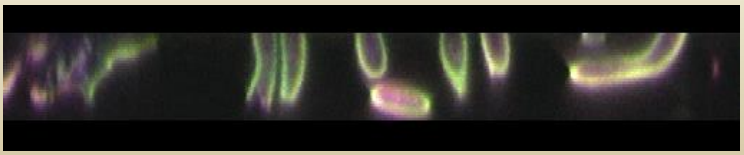


xz

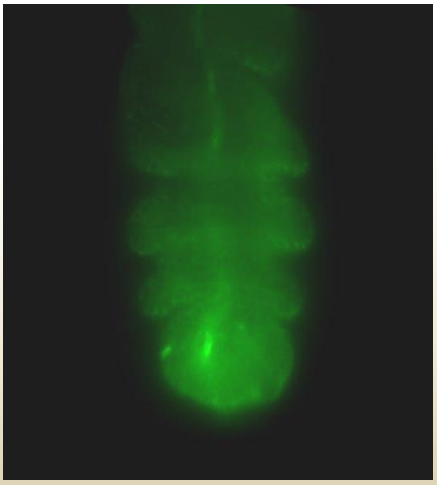
Convallaria

Starch grain
Cell wall





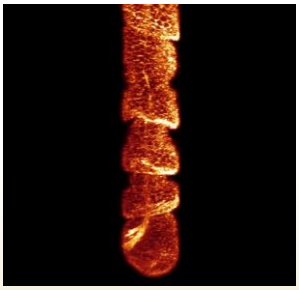
xyz scanning

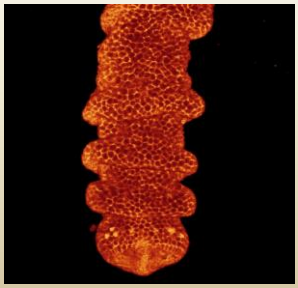


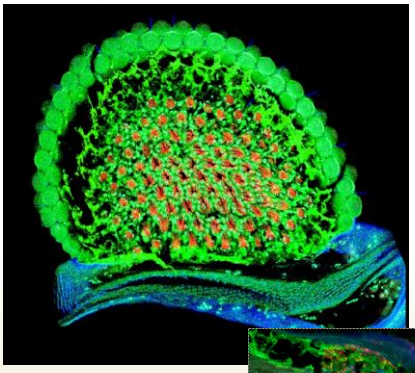
Drosophila leg, FITC, non-confocal

confocal

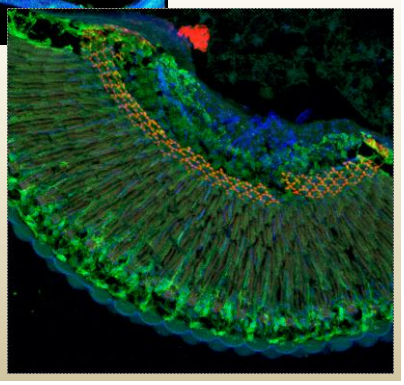
xyz





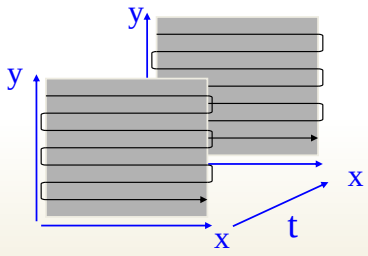


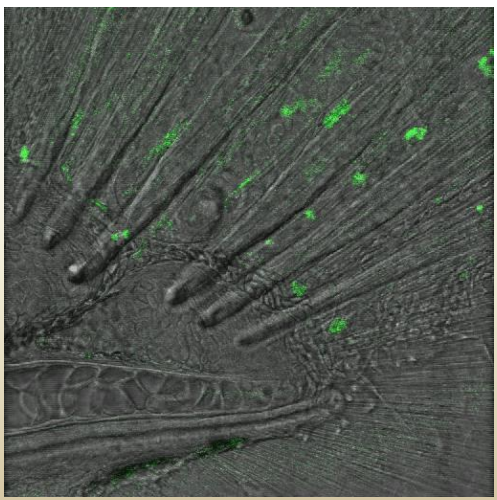
Drosophila melanogaster, eye section
Red: F-Actin, Cy3
Blue: Nuclei, DAPI
Green: pigmented cells, GFP
Courtesy of Anne Galy, IGBMC, Strasbourg-Illkirch, France



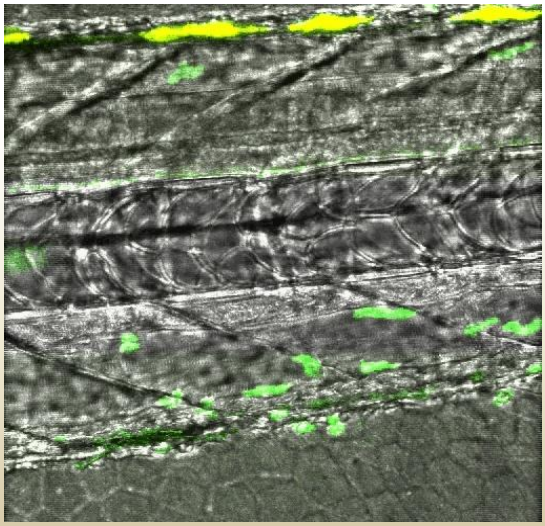
Drosophila melanogatser, eye section
Red: F-Actin, Cy3
Blue: Nuclei, DAPI,
Green, Retina cells, GFP
Courtesy of Anne Galy, IGBMC, Strasbourg-Illkirch, France

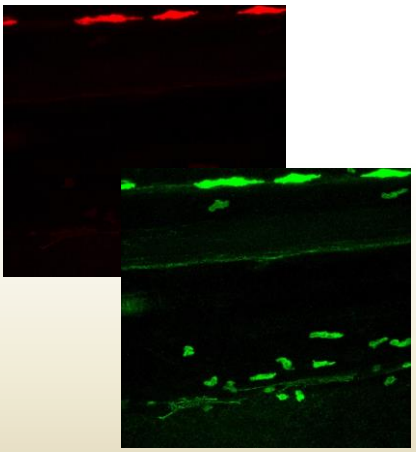
xyt





xyzt



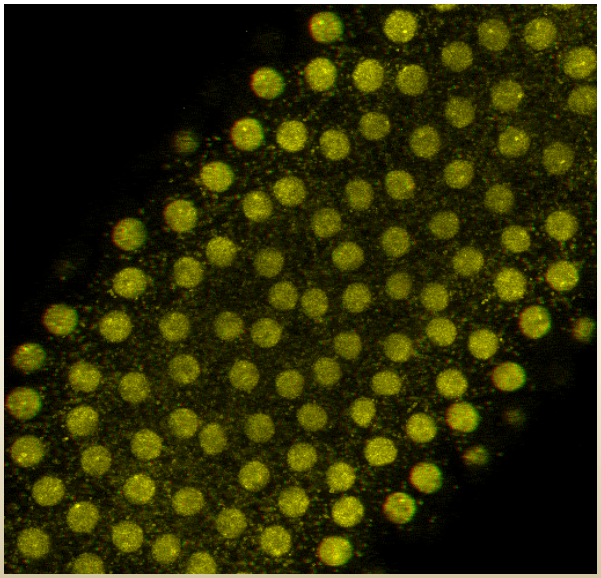


Fish embryo (Medaka)
Flow of red blood cells and migration of macrophages.
Macrophage: YFP and RFP (1st & 2nd ch)
Red blood cells: TLD

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

Multidimensional
Confocal Imaging

xyzt

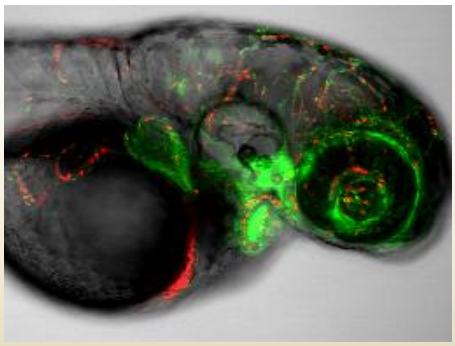


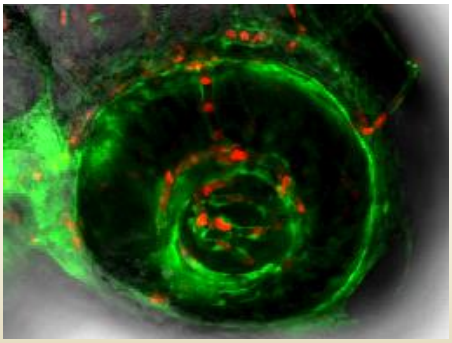
Drosophila mitosis
pre-cellularized embryo
GFP-tagged histone,

400 frames**,** 20 sec
between each stack,
each frame presents
a stereo image

Courtesy:
Prof. Sullivan (University of California, Santa Cruz);
Robert Saint (Adelaide, Australia)

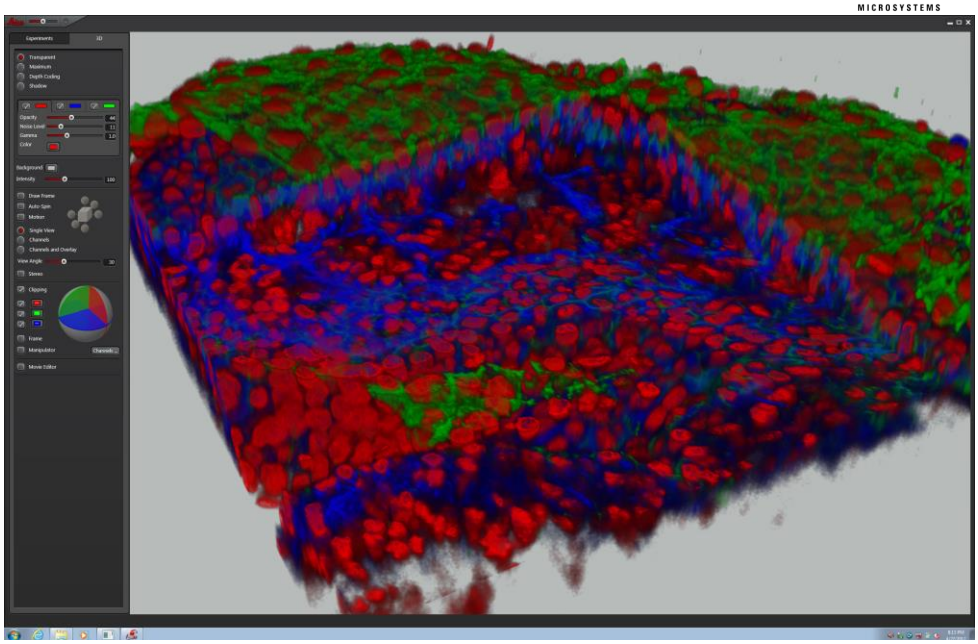
xyzt





LEICA TCS SP5 AOBS
- 中研院細生所 -

LAS X 3D



Ready to grow further

STED

HCS-A

WLL

SMD

MP

CARS

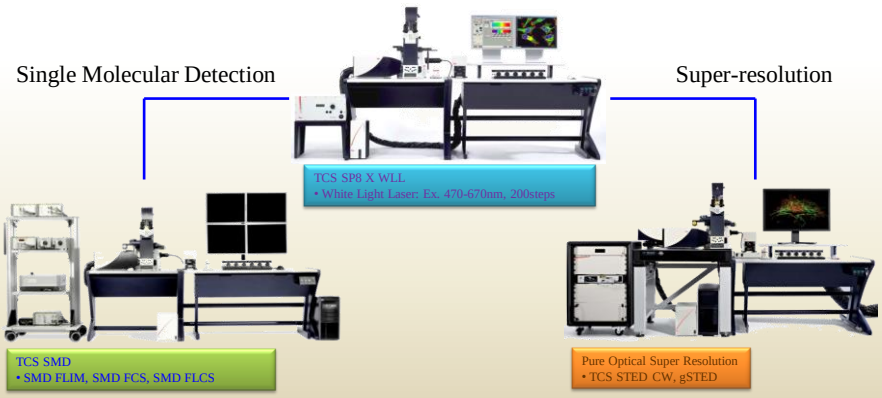
CFS

共軛焦顯微鏡的發展趨勢：

Confocal system is not only for confocal images, but a platform for new application development…

Single Molecular Detection

Super-resolution



FLIM-FRET
Mini environment
Binding…

Pure optical resolution < 50nm

Yes, our TCS SP8(X) is ready…

Superresolution- STED

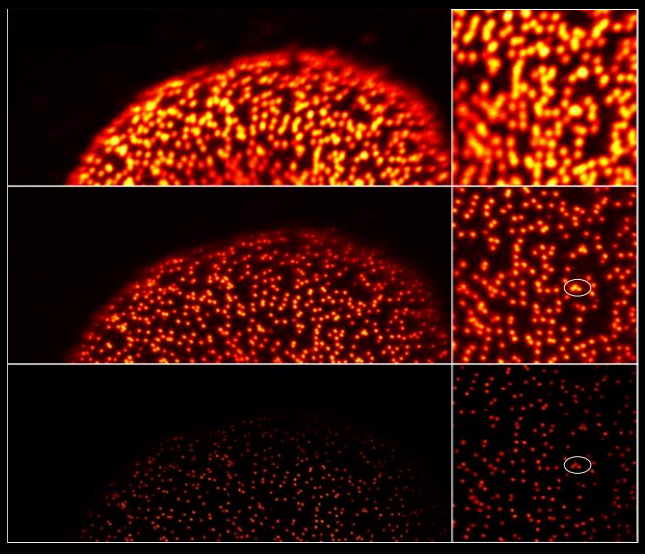
Sub 50nm resolution specified!

IF: NUP complex; Alexa 488

Confocal

STED CW

Gated STED



Leica MICROSYSTEMS

Thank you

Major Instruments Co., Ltd.