Travel to New Dimensions- LSM 880

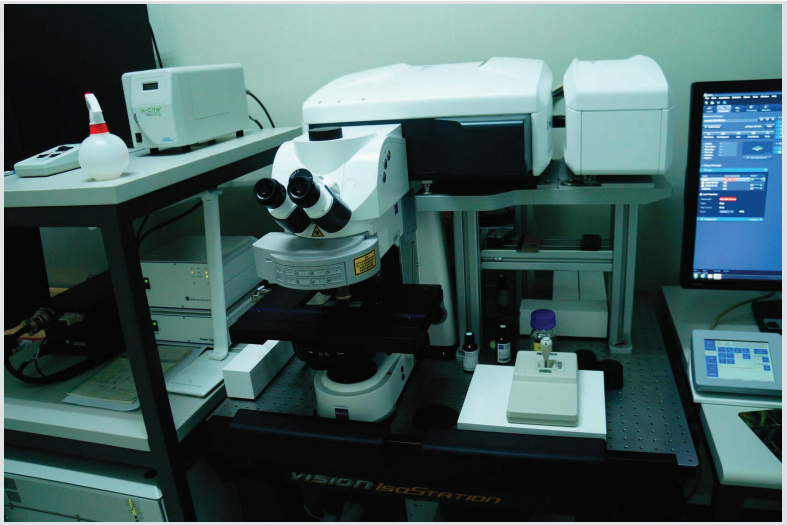
Sensitivity,
Flexibility and
Ease of Use

Innovative High-End
Laser Scanning Microscopes
from Carl Zeiss

余雅倩
台灣儀器行

LSM 880: The Power of Sensitivity

Our Latest Member of the LSM 880 with GaAsP Detectors



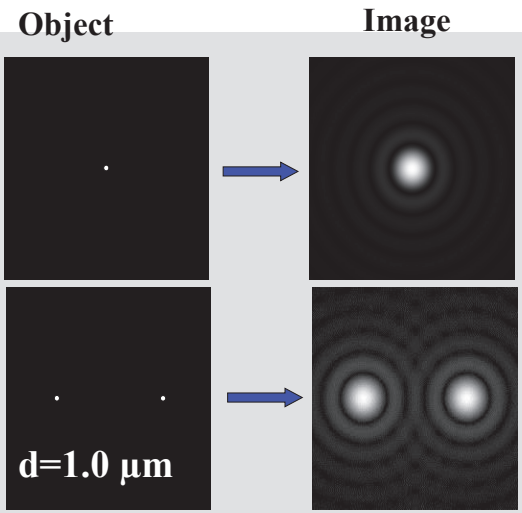
The Resolution of a Microscope is limited

What does that mean?

The image of a point-like structure is not a point, but a diffraction pattern with a finite extension.

This 2-dimensional pattern in the image plane is also called the Airy-disc.

In general, the image of a point-like structure is called the Point Spread Function (PSF).

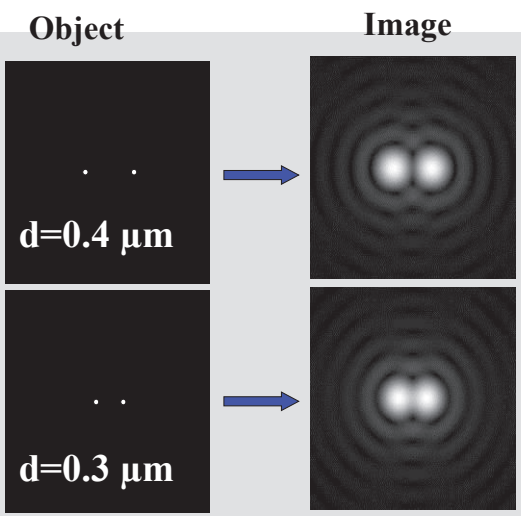


The Resolution of a Microscope is limited

Definition

The resolution limit is reached, when two point-like objects can not be imaged as two distinct structures anymore.

The distance between the objects is called the resolution limit.



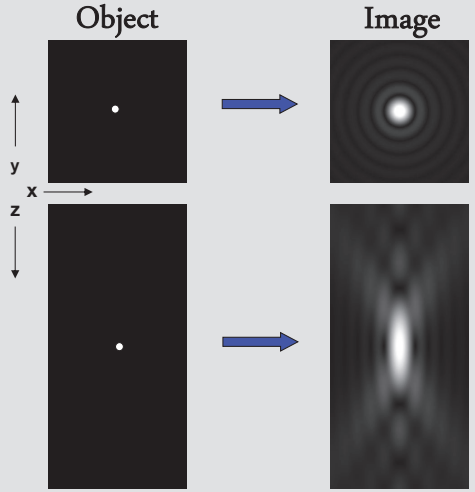
The Point-Spread-Function is a 3-dimensional function

.

The axial shape of the PSF is completely different from the lateral one.

The axial extension is larger than the lateral.

→A microscope has a lateral and an axial resolution.



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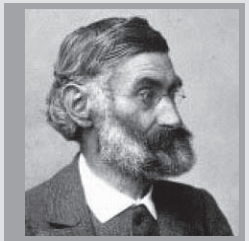
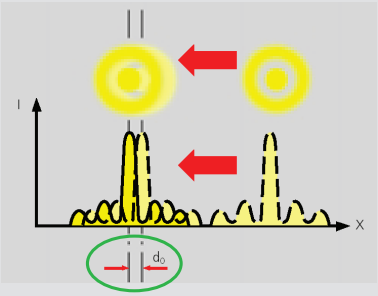


Prof. Ernst Abbe
(1840 - 1905)

$d = \frac{\lambda}{2n\sin\alpha}$ (1876)



$d_0 = \frac{1.22\lambda}{N.A_{obj.} + N.A._{Cond}}$ or more simply $d_0 = \frac{\lambda}{2N.A}$

λ = wavelength of light, e.g. 550 nm (green)

The Resolution of a Microscope depends on Numerical Aperture and Wavelength

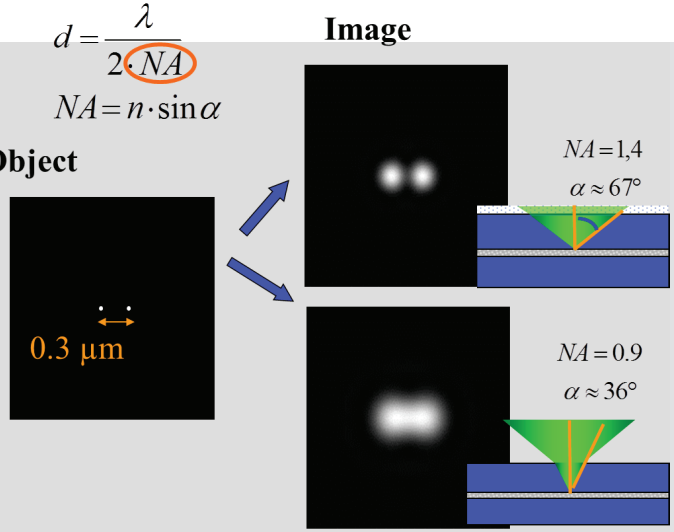
$$d = \frac{\lambda}{2 \cdot NA}$$

$$NA = n \cdot \sin\alpha$$

Principle effect

High numerical aperture objectives have a large opening angle.

The higher the NA, the better the resolution of the microscope.

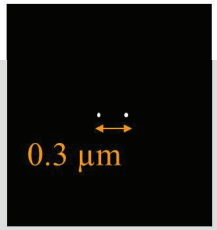


The Resolution of a Microscope depends on Numerical Aperture and Wavelength

Priciple effect

Shorter wavelengths generate smaller Point Spread Functions.

The shorter the wavelength, the better the resolution.



$$d = \frac{\lambda}{2 \cdot NA}$$

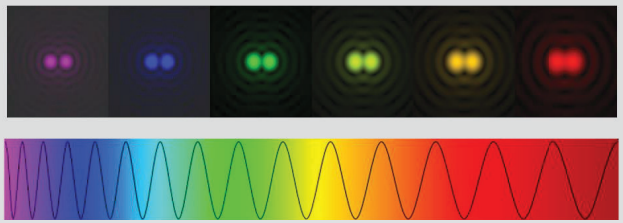
$$NA = n \cdot \sin\alpha$$

$NA = 1{,}4$

$\alpha \approx 67°$

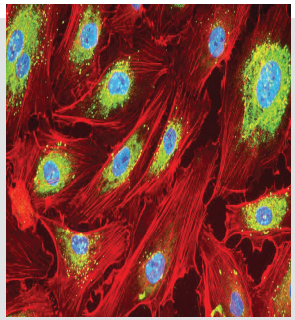
Image

λ = 350nm 480nm 520nm 570nm 610nm 640nm



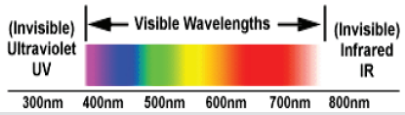
Different Beam Path of Image Formation

Fluorescence -- Wavelength of visible light



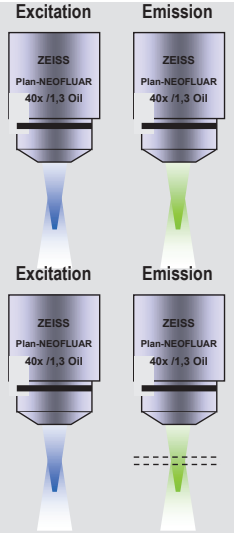
Human endothial cells with 3 fluorescence markers: Actin (Phalloidin/TRITC), von Willebrand Factor (Oregon-green), cell nucleus (DAPI).

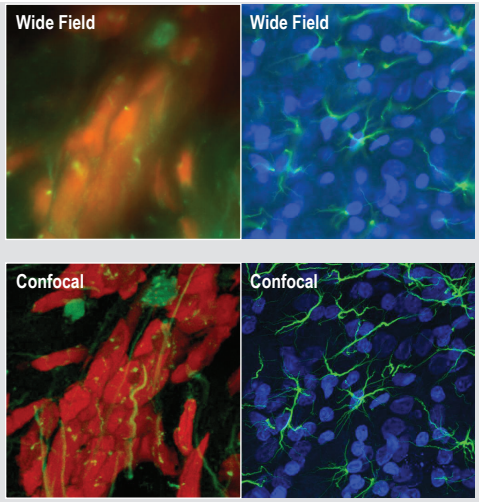
Wavelength color	
1. 340 - 400 nm near Ultraviolet (UV) - Invisible	
2. 400 - 430 nm	Violet
3. 430 - 500 nm	Blue
4. 500 - 560 nm	Green
5. 560 - 620 nm	Yellow to Orange
6. 620 - 700 nm	Orange to Red
7. Over 700 nm near Infrared (IR) - Invisible	



Confocal Laser Scanning Microscopy

Optical sectioning: elimination of out-of-focus light





The Comparison Between the LSM and the Conventional Light Microscope

	Wide Field Microscope	Laser Scanning Microscope
Light Source	**Mercury or Xenon Lamp**	**Laser**
Illuminated Field	**Wide Field**	**Spot**
Image Acquisition	**Parallel, Frame at Once**	**Sequential, Pixel wise**
Signal Separation	**Dichroic Beam Splitter, Emission Filter**	**Beam Splitter Cascade, Emission Filter**
Detector	**Eye or CCD Camera**	**Diffraction limited by pinhole →Photomultiplier (PMT)**

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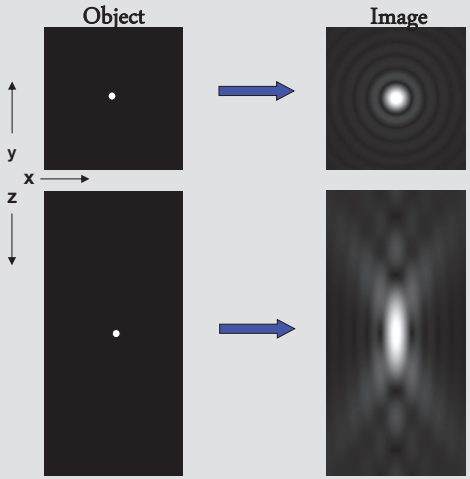
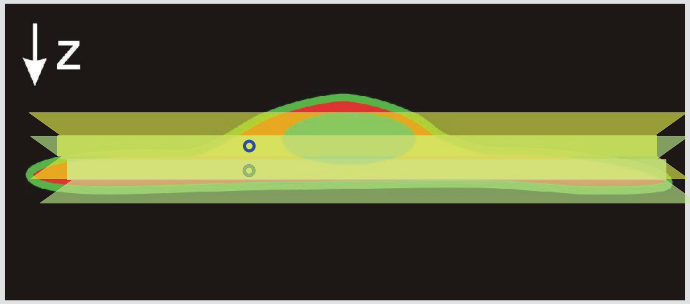


Conventional/Widefield Fluorescence

Background emission from deeper image planes



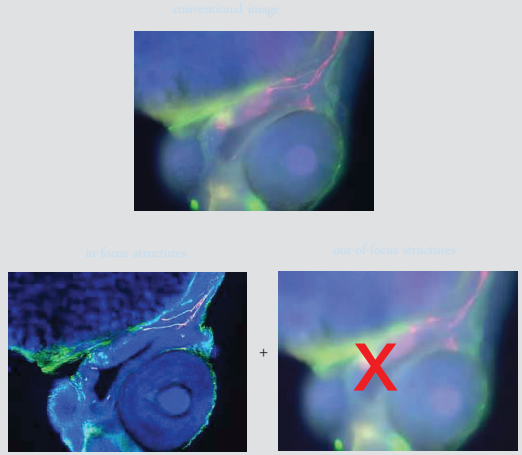
Structures which are „out-of-focus“ become visible in conventional widefield-fluorescence. Because of the focal depth inherent in all objectives, they are visible as an image blur (haze, image fog).

Why do we need Optical Sections ?

The Fundamental Problem

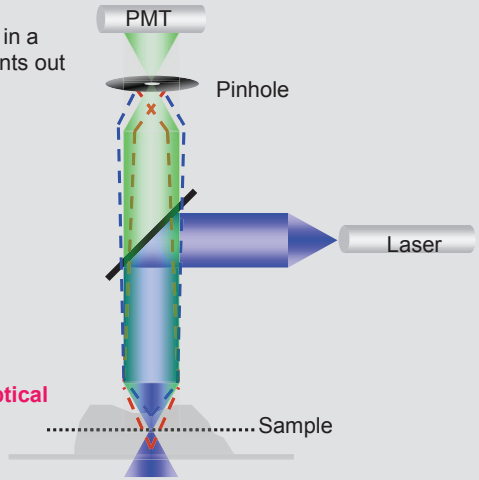
Conventional Images

Conventional images from 3-dimensional objects consists of light from structures, which are in focus and the light from structures which are not in focus.



The confocal principle

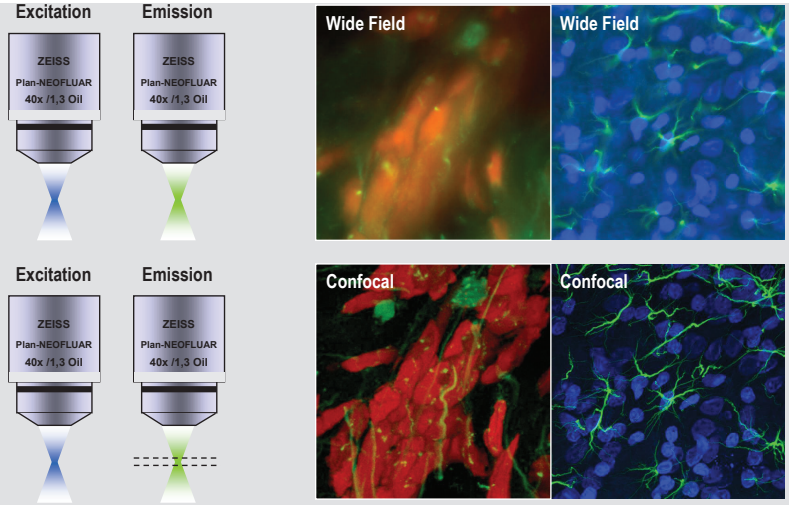
A minute diaphragm, situated in a conjugated focal plane, prevents out of focus light to be detected.



The pinhole diameter directly controls the thickness of the **optical section .**

Confocal Laser Scanning Microscopy

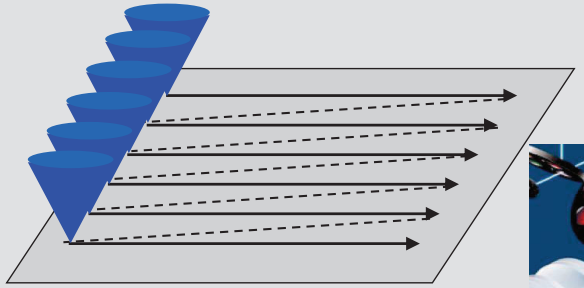
Optical sectioning: elimination of out-of-focus light



Confocal: Point Scanning

From Spot to Image

- To get a 2 dimensional image from the specimen, the excitation spot has to be moved over the specimen
- The scanning mirrors move the excitation beam in a line wise fashion

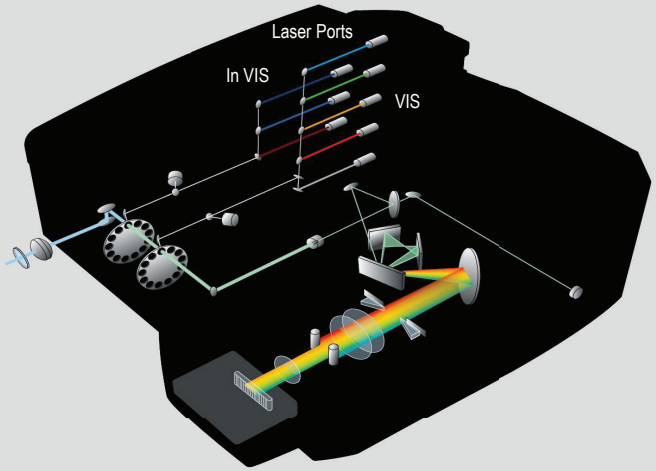


XY scanning

Point scanning confocal systems

LSM 710/780/880

Innovative Beam Path Technology



3 Channel Spectral with one GaAsP Detector

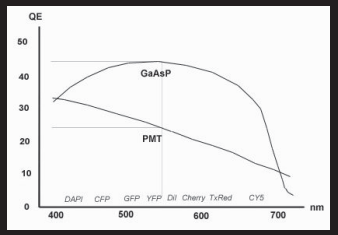
Unmatched sensitivity

GaAsP (*Gallium Arsenide Phosphide*) is a semiconductor material with ideal characteristics for converting photons into electrical signals.

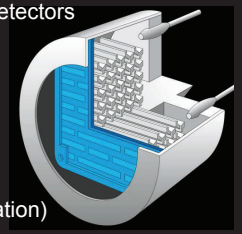
Benefits of GaAsP detectors:

Almost two times better SNR than PMTs (resulting in higher sensitivity, better image quality and higher acquisition speed).

GaAsP detectors can be operated in integration mode as well as in photon counting mode.



Typical sensitivity of detectors



GaAsP detector
(schematic illustration)

Get More Results With GaAsP Detectors

Applications Benefit from Improved Sensitivity in Many Ways

Better image quality

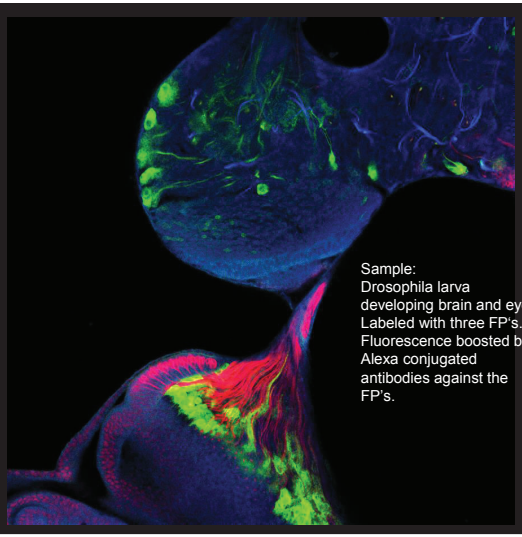
- Higher sensitivity equals better signal-to-noise ratio (detection of faint signals)

Faster scanning

- Data recording at shorter pixel times
- Need for averaging strategies largely reduced
- 13 fps

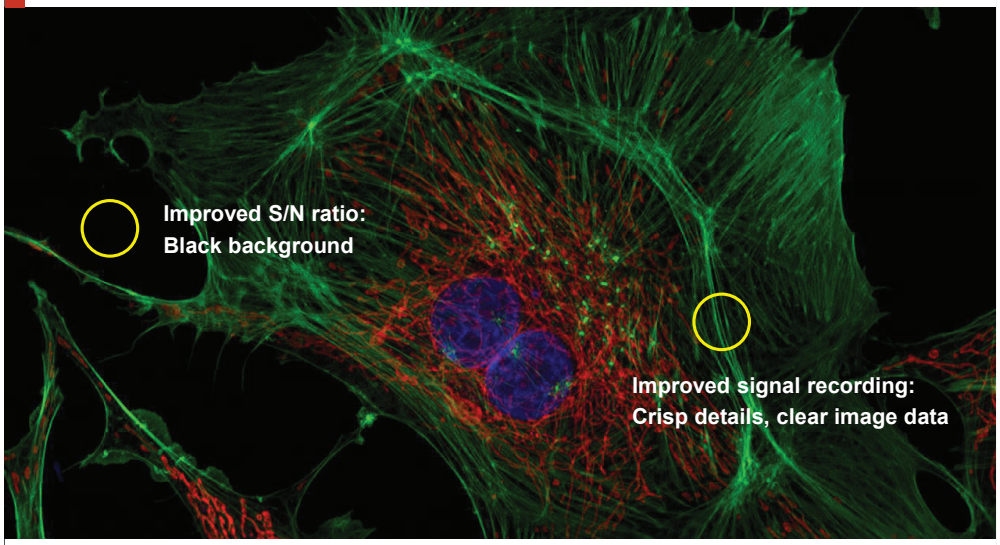
Acquisition of more data

- Data recording at lower laser power (reduced bleaching and photo-toxic effects in live cell imaging)

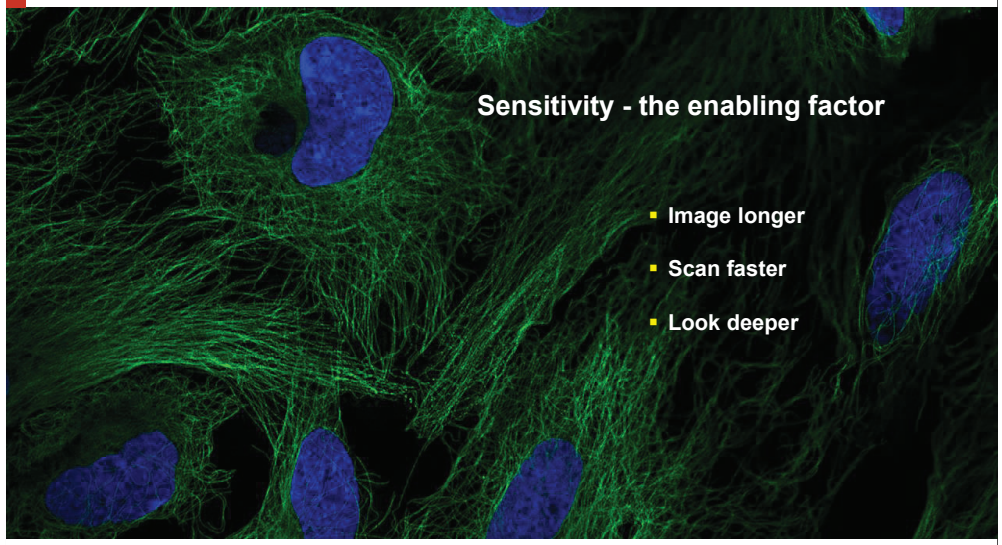


Sample:
Drosophila larva developing brain and eye. Labeled with three FP's. Fluorescence boosted by Alexa conjugated antibodies against the FP's.

LSM 880
The power of sensitivity



LSM 880
The power of sensitivity



LSM 880
Laser line

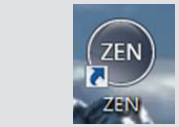
Laser line	Fluorochrome
405 nm	DAPI, Hoechst, Alexa 405, BFP
458 nm	ECFP
488 nm	Alexa 488, Fluo-4, FITC, eGFP
514 nm	EYFP
561 nm	Rhodamine, Alexa 546, 555, 568, Cy3, TRITC, DsRed, Texas Red, MitoTracker Red, mCherry
633 nm	Alexa 633, Cy5

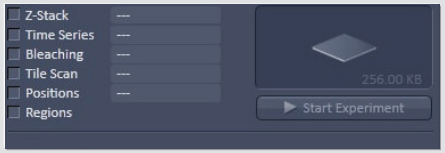
Detectors:
QUASAR Detection (3) for fluorescence images

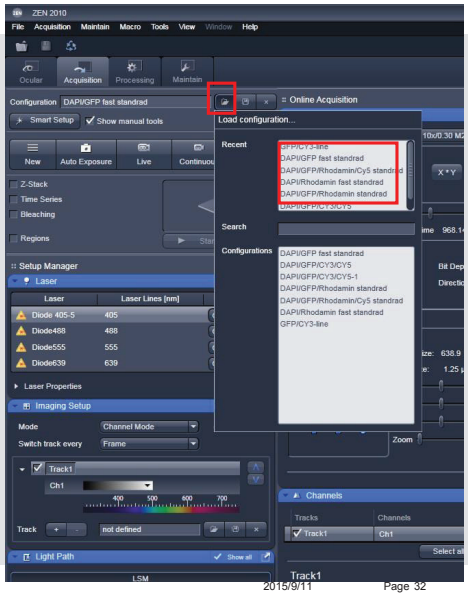
1 transmitted PMT detector for Bright Field (PH/DIC) images

ZEN 2 - Efficient Navigation
Powerful software for powerful LSM systems

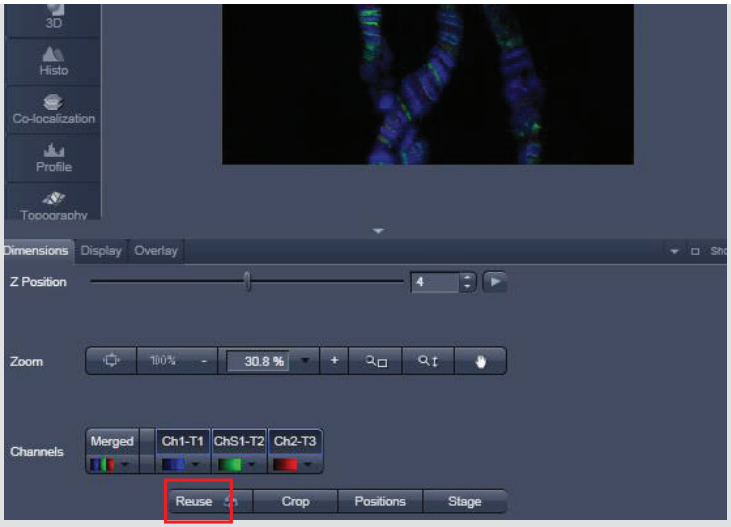
ZEN 2
Load configuration





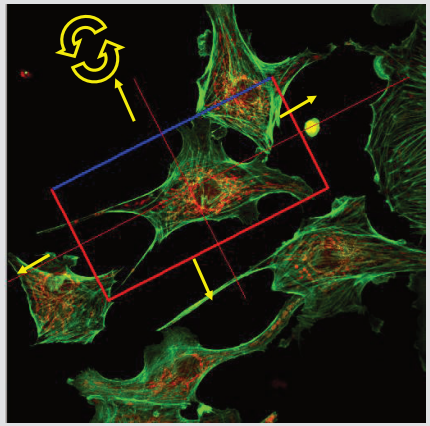


ZEN 2
Reuse function: recur all parameters and setting

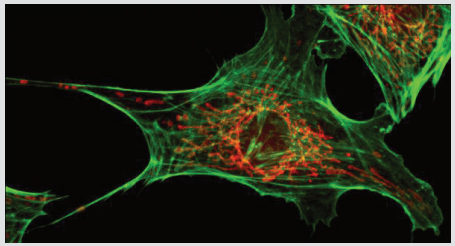


Major tasks of a LSM
Laser and scanning mirror control

Two independent scanning mirrors

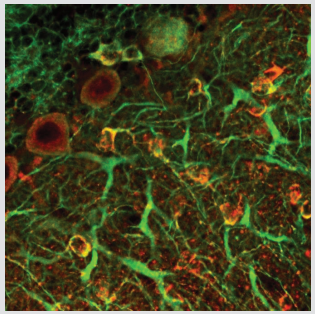


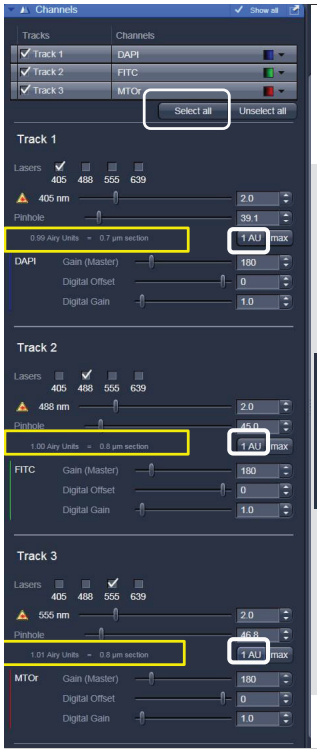
Free scan field rotation (0-360º)
Free online zooming (0.6~40x (zoom=66.7x)
Any geometry: 1x4... 6144*6144
Faster rectangular acquisition (e.g. video rate)



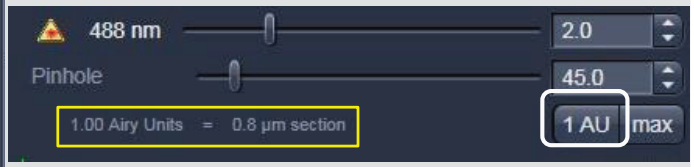
Major tasks of a LSM
Colocalization in Confocal Microscopy

- Acquisition of Crosstalk free images required
- Occurrence of two fluorescent emission signals inside the same detection volume
- Identical size of detection volumes for different color channels required
- Intensities and position of the signals inside the detection volume may vary



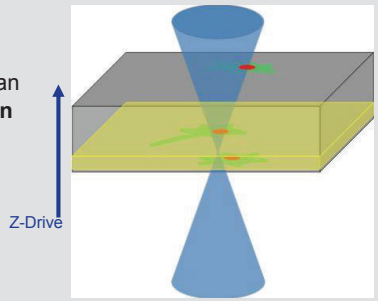


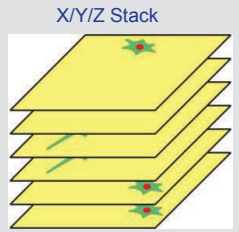
Select all
1 AU或調整pihhole至相同的光學切片厚度



Major tasks of a LSM
Optimal optical sectioning in thick tissue Z stack

This plane represents an **optical section**

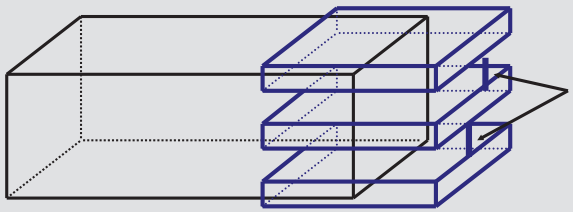




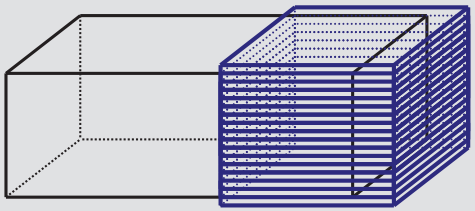
- 3 D information is acquired by moving the excitation focus not only in XY direction but also in Z direction
- The result is a **3 D data stack** consisting of number of XY images representing different optical sections from the specimen

Major tasks of a LSM
Optimal optical sectioning in thick tissue Z stack

Number of sections



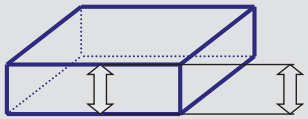
Missing Information



Sample bleached and much data,

Major tasks of a LSM
Optimal optical sectioning in thick tissue Z stack

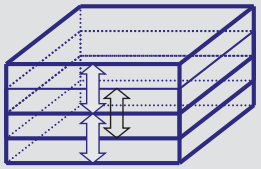
Optimal Number of sections : no missing information at minimal number of sections

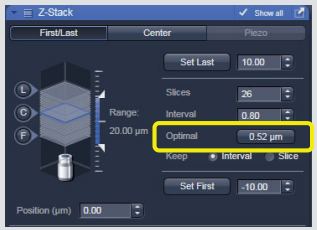


Optical thickness depends on:

- wavelenght l
- objective lens, N.A.
- refractive index n
- pinhole diameter P

$d \sim P\ n\ l\ /\ (N.A)^2$



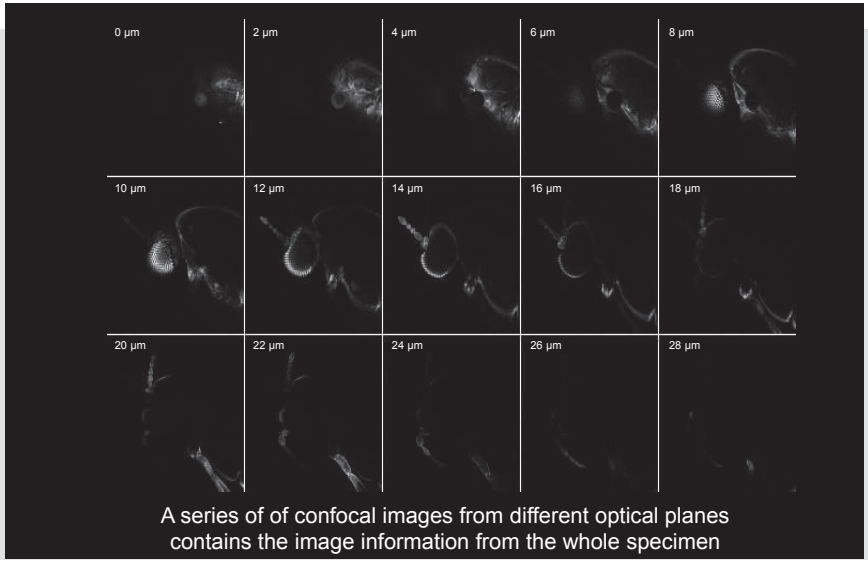


„Nyquist-“ or Sampling- Theorem
slices overlap by the 50% of their thickness

LSM software: One click for best resolution

Flexibility

Major tasks of a LSM
Optimal optical sectioning in thick tissue Z stack



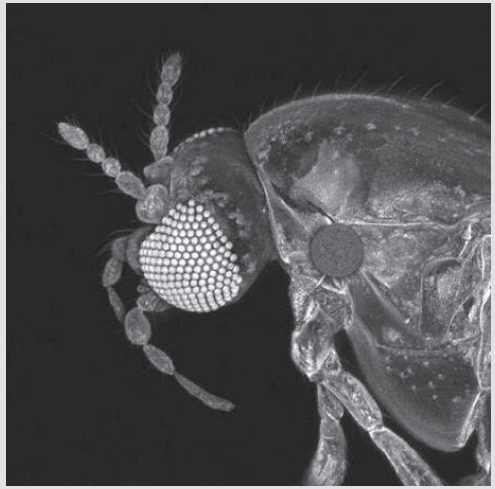
A series of of confocal images from different optical planes contains the image information from the whole specimen

Flexibility

Major tasks of a LSM
Optimal optical sectioning in thick tissue

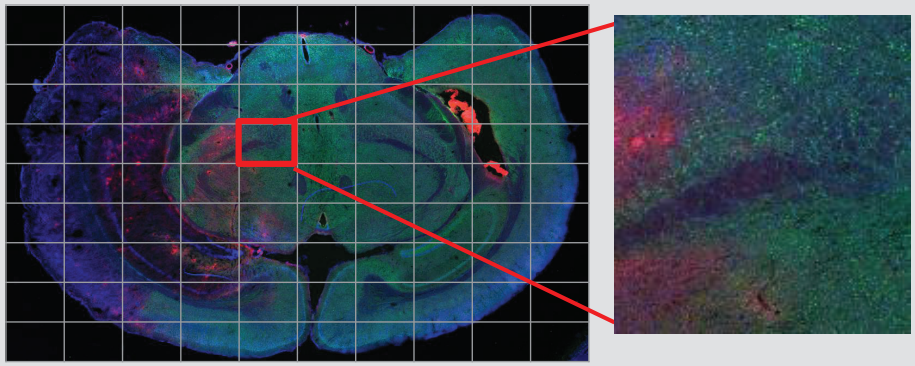
- An overlay (maximum projection) of these single images results in an image with an **enhanced depth of focus**
- This image contains all information from the specimen

Every detail is in focus !



Flexibility

Tile scanning with motorized scanning stage
大面積高倍數掃描



40X objective, 10X9

Versatile

LSM 880 – Get More Results !
Innovative High-End LSMs from Carl Zeiss

Sensitiviy

- Extremely light-efficient instrument design
- New super-sensitive GaAsP detectors for LSM 880

Flexibility

- QUASAR detection unit allows for maximum flexibility in signal recording
- Modularity: Configuration of sophisticated imaging platforms through integration of LSM with additional detection modules

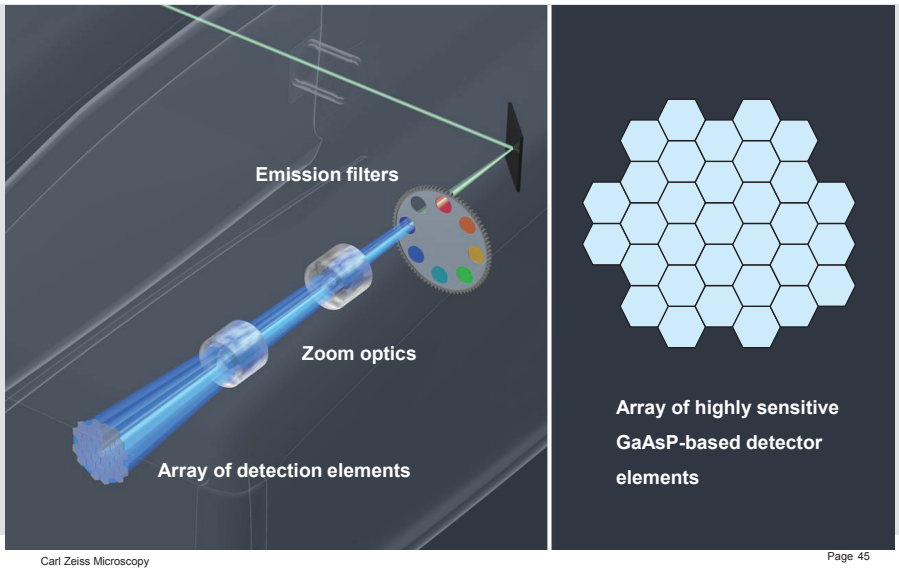
Ease of Use

- ZEN 2: Powerful software for sophisticated LSM applications
- User-friendly graphical interface

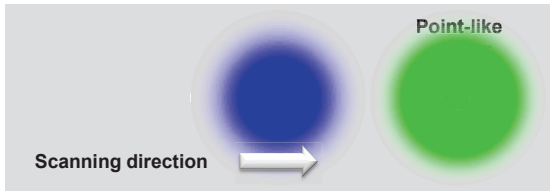
LSM 880: The Power of Sensitivity

Our Latest Member of the LSM 880 with GaAsP Detectors

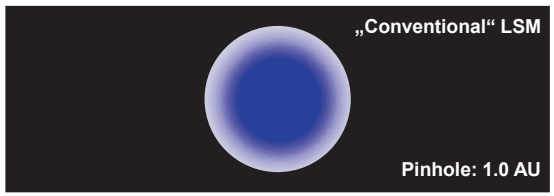
Airyscan introduces a revolutionary new concept designed to overcome a “classical limitation” of LSMs



In practice, confocal imaging is mostly a compromise that tries to balance resolving power and SNR



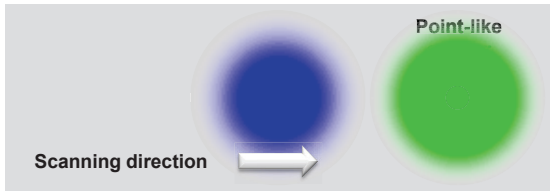
Fixed registration of excitation spot (blue) and detection unit



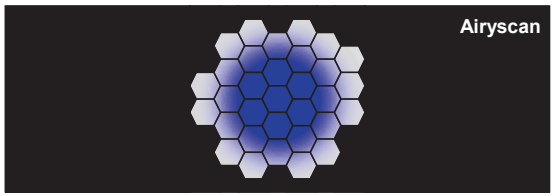
The Problem:
The resolving power of LSMs stays far below its potential maximum when setting the confocal pinhole to 1 AU.

Note:
The signal-to-noise ratio (SNR) is acceptable if the pinhole is set to 1 AU.

Airyscan overcomes a “classical limitation” of LSMs with its arrayed detector elements all utilized in parallel



Fixed registration of excitation spot (blue) and detection unit



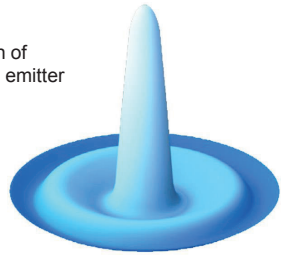
Solution:
Array of detection elements

Benefits:
Improved SNR (utilizes light otherwise rejected at small pinhole diameters) and additional spatial information about the signal!

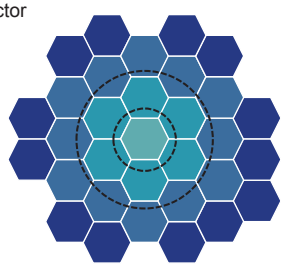
Note:
Each detector element compares to a confocal pinhole set to 0.2 AU („sub-Airy sampling“).

In brief: Airyscan takes advantage of spatial information not recorded with “conventional LSMs”

Airy pattern of
a point-like emitter



Array detector
of Airyscan



The offset of individual detectors to the optical axis provides **additional spatial information** in Airyscan (detectors of a „conventional“ LSM just integrate all light passing through its pinhole).

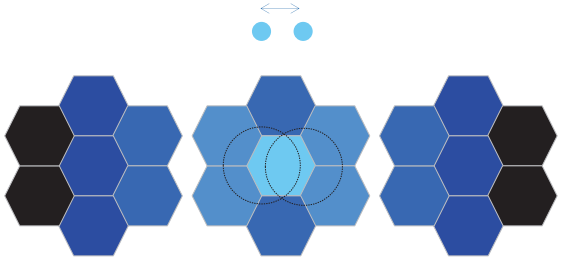
Linear deconvolution assigns all signals (and frequencies) recorded by individual detector elements to their appropriate locations.

Result:
Isotropic 1.7-fold increase in resolving power!

(Further reading: White paper on Airyscan)

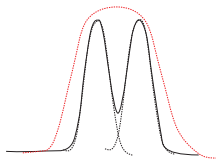
LSM 880 – Airyscan detects intensity distribution

Narrower PSF means improved resolution



Consider intensity
Consider distribution

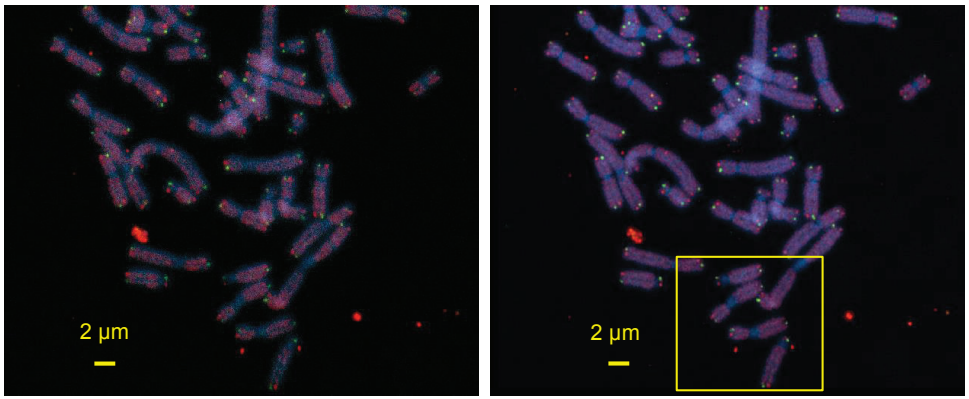
Scan



Both objects that were indistinguishable now become resolved in space.

LSM 880 – Airyscan enhances resolution, boosts SNR

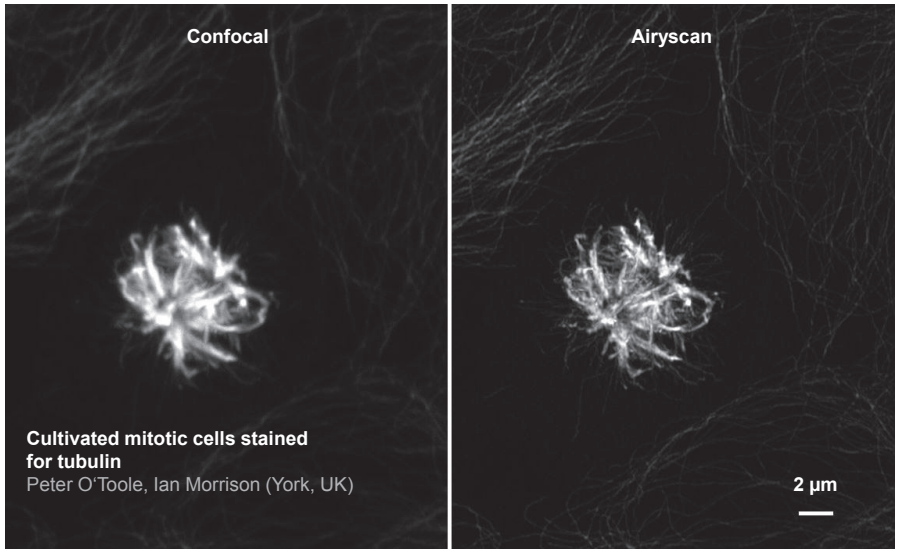
“...thereby allowing for a much more accurate quantification“
(Karlseder and Fitzpatrick, The Salk Institute, La Jolla, CA, USA)



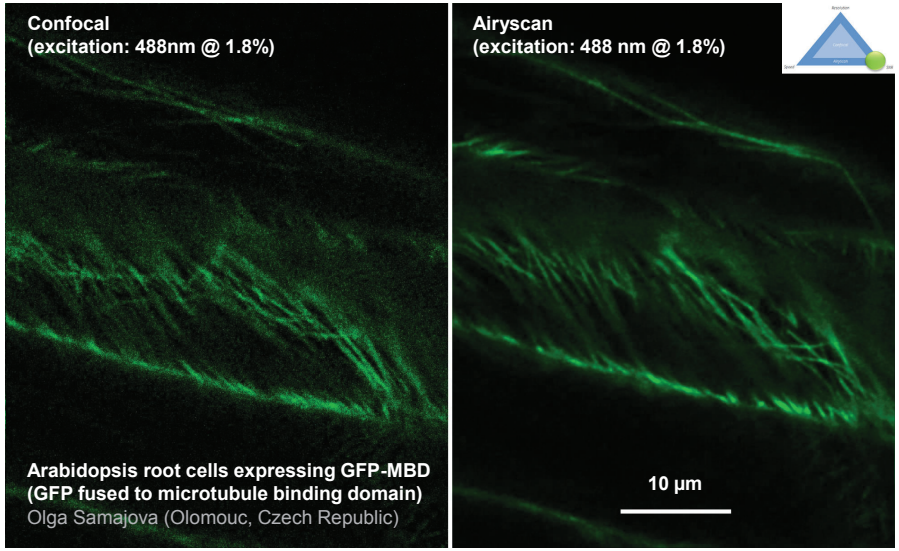
Telomere replication without RTEL1: Stalled forks and telomere breakage visualized as doubled dots using Airyscan. Resolution is meaningless without good SNR.

Courtesy: J. Karlseder Ph.D. (Molecular and Cell Biology Laboratory) and J. Fitzpatrick Ph.D. (Director, Waitt Advanced Biophotonics Core), The Salk Institute, La Jolla, USA.

Airyscan reveals more details in your samples by increasing the resolution of LSM up to 1.7-fold

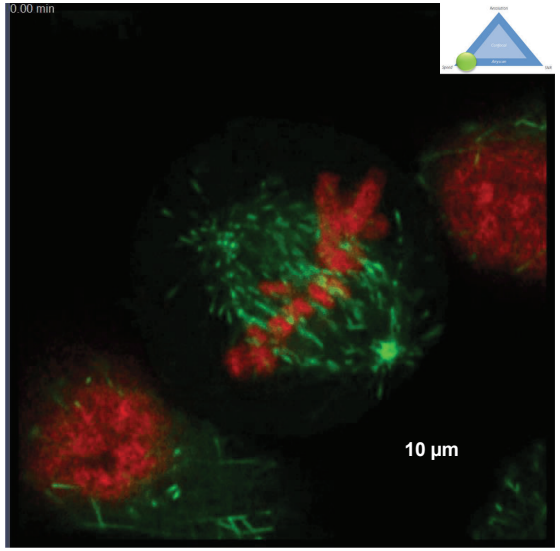


With its drastically improved SNR, Airyscan delivers quality images previously impossible with LSMs



Arabidopsis root cells expressing GFP-MBD (GFP fused to microtubule binding domain)
Olga Samajova (Olomouc, Czech Republic)

Airyscan delivers exceptional data of live samples using the same laser power than in confocal imaging

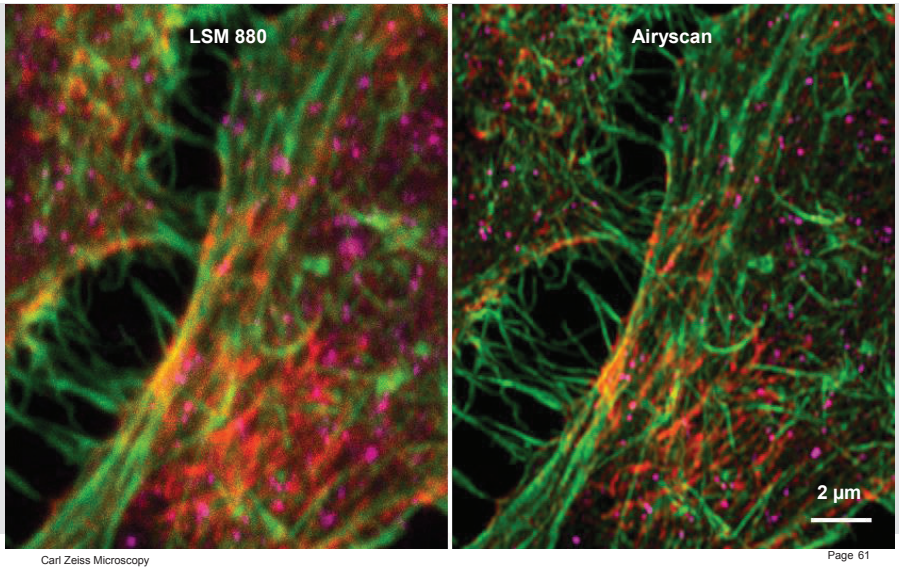


Mitosis in HeLa-Kyoto cell line during mitosis. Imaged with ZEISS LSM 880 / Airyscan.

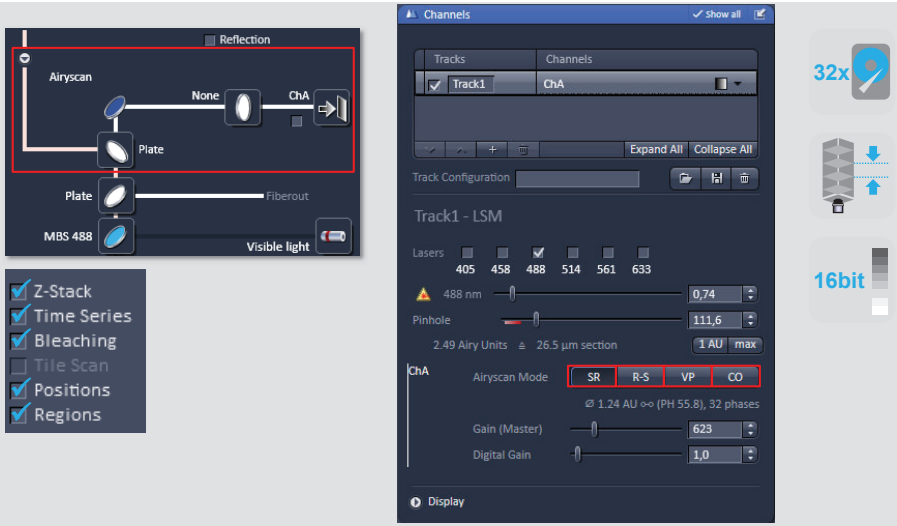
Video showing Histone 2B (H2B, red, mCherry) and microtubule end-binding protein 3 (EB3, blue, EGFP)

Sample courtesy of:
Jan Ellenberg,
EMBL, Heidelberg.

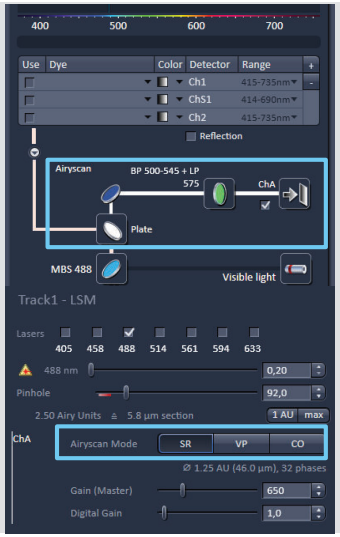
Airyscan performs multi-color imaging of samples stained with up to four fluorescent labels

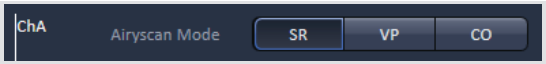


Airyscan: Software Integration



LSM 880 with Airyscan: Easy of use
3 different modes of Airyscan detector



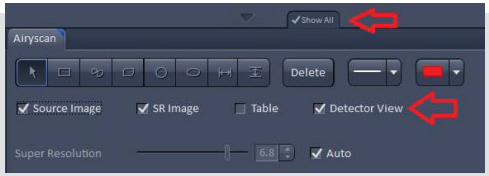


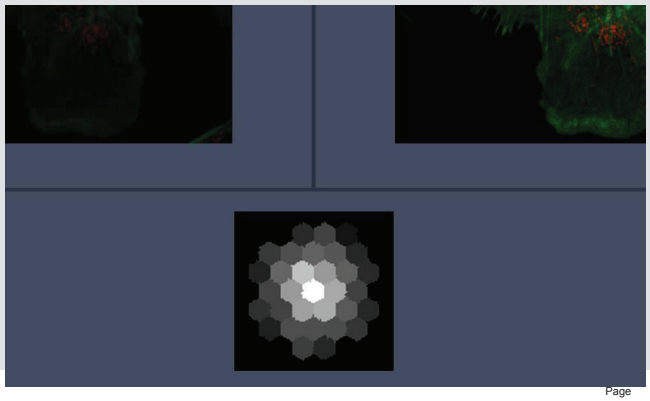
SR: Superresolution (up to 1,7 fold) using the Airyscan detector to produce effectively small pinholes. Oversampling and deconvolution are used to generate images with up to 140 nm resolution in xy and 400 nm in z.

VP: In virtual pinhole mode images are collected with an open (> 3 A.u.) pinhole. Using the distribution on the array – pinhole can be adjusted as needed in a post acquisition step.

CO: Confocal mode just uses the sum total signal from the array, using it as a single extra channel.

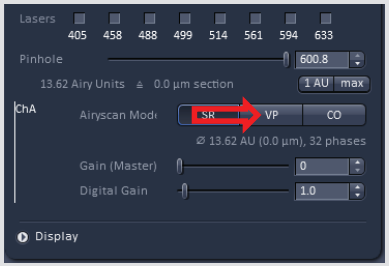
LSM 880 with Airyscan: Easy of use
SR Mode Detector View



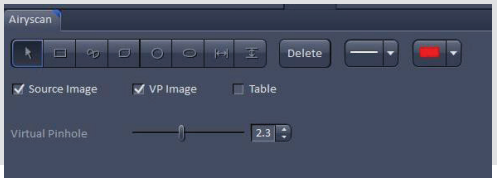


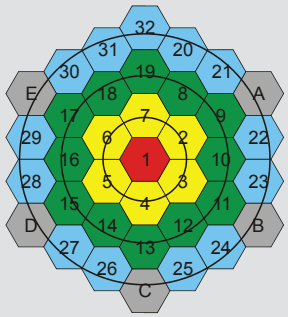
LSM 880 with Airyscan: Easy of use
VP Mode

Select “VP” mode BEFORE acquisition



In the Airyscan processing tab, instead of SR strength, the software display VP parameters (1-4 AU)





	LSM 510 Meta	LSM 880 / Airyscan
Data depth	8, 12 bit	8, 12, 16 bit
視野大小	18 mm	20 mm
光學變焦	0.7x~40x	0.6x~40x
掃描速度	5 fps (512x512)	13 fps (512x512)
光譜分析	解析度~10 nm	解析度~3 nm
穿透光感測器	穿透光 T-PMT	穿透光 T-PMT
反射光訊號	無	感測器可接收反射光的訊號
軟體	AIM	ZEN
雷射搭配	Ar laser (458, 477, 488, 514nm); HeNe laser 543nm; HeNe laser 633nm; Diode laser 405nm	Diode laser 405nm; Ar laser 458, 488, 514nm; DPSS-laser 561nm; HeNe laser 633nm;
應用	一般玻片樣品confocal 掃圖與分析	高感度GaAsP感測器，與高超解析度(SR, VP, CO mode)

Thank you for your attention!!