

## **ZEISS LSM 9 Family with Airyscan 2** Multiplex Mode for Fast and Gentle Confocal Superresolution in Large Volumes

# ZEISS LSM 9 Family with Airyscan 2

## Multiplex Mode for Fast and Gentle Confocal Superresolution in Large Volumes

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### Introduction

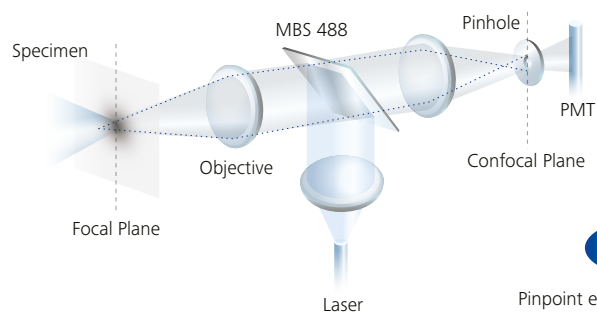
Life science researchers seek to describe and characterize a condition, malady or biological phenomena to ultimately provide a cure, therapeutic treatment or other advancement. How well a finding translates into a useful therapy for humans often depends on how well the findings that occur in the chosen model system scale up to human or other systems. Further the ease of which a model system is accessible for alterations or manipulations to replicate the phenomena of interest often dictate the choice of model system in biomedical research. Confocal microscopy is one important research tool that provides the ability to observe a model system in its natural state while providing specificity in labelling structures and or molecular sensors to report biological function to quantify the perturbation within the desired model system. Hence the choice of model system and application dictates the required capabilities of a confocal microscopy solution.

Desired for their optical sectioning capabilities, confocal microscopy systems have been commercially developed to become highly flexibly imaging tools accommodating most imaging applications and common model systems. However, like most research tools, inherent performance limitations owed to the current technology, limit the tools application. Traditional confocal microscopy is no different. With new molecular biology tools pushing protein expression levels lower, availability of new 3D model systems, transition to small organisms, and the growing need to study structures beyond the diffraction limit, the once sought-after image formation flexibility of confocal microscopy is at its limit.

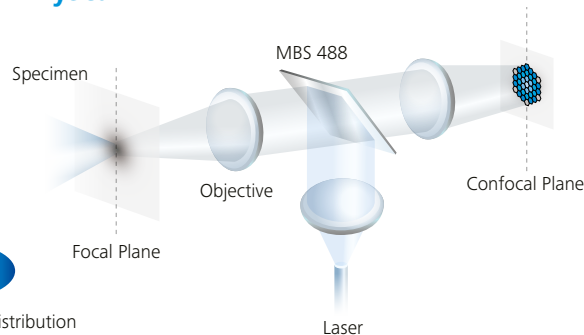
With the launch of the LSM 8 family with Airyscan, ZEISS revolutionized the traditional confocal system by replacing the conventional pinhole/PMT detector arrangement with an innovative pinhole-plane imaging concept. The Airyscan detector design from ZEISS extracts much more of the information from the fluorescence signal that resides at the pinhole plane affording researchers simultaneous increases resolution and signal-to-noise while still providing improved optical sectioning capabilities. Subsequent to the initial Airyscan launch, ZEISS has continued to develop the Airyscan detection concept providing higher acquisition speeds and thinner optical sections, e.g. Fast mode for Airyscan and 2D superresolution mode.

The LSM 9 family with Airyscan 2 represents the next big step in the evolution of confocal microscopy. With a focus on usability, utility and throughput, the Airyscan 2 detector from ZEISS fully leverages current electronics to create new data handling schemes. The net result of these innovation steps is an upgraded user experience offering a 6.6x reduction in data size and 5x reduction post processing times (1024x/1024y/150z image dimensions) all while maintaining the simultaneous 4x-8x increase in image SNR and super-resolution (120nm x/y, 350nm z). In combination with the Airyscan 2 improvements, the principal focus for Airyscan 2 is the new Multiplex mode. Through new acquisition strategies, the new mode empowers researchers to leverage the unique combination of optically sectioned superresolution and sensitivity at the highest volume rates.

## Traditional Confocal



## Airyscan 2



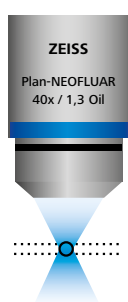
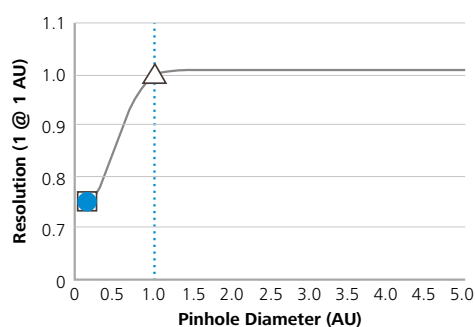
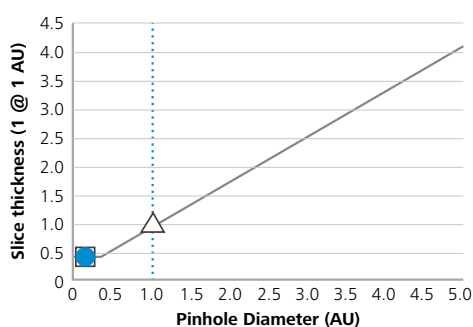
**Figure 1** (Left) Schematic of a traditional laser scanning confocal microscope with a pinhole/PMT arrangement. (Right) Schematic showing the placement of the Airyscan 2 detector in the pinhole plane acting as a series of small 0.2 Airyunit (AU) pinholes providing improved optical resolution with the collection ability of a 1.25 AU pinhole.

The Multiplex mode innovation for Airyscan 2 focusses on the need to capture structural dynamics, cellular signaling, molecular trafficking and diffusion events with real-time superresolution and superior SNR in model systems that extend beyond traditional cultured cell preparations and into new 3D model systems of organoids, spheroids or 3D culture and whole organisms.

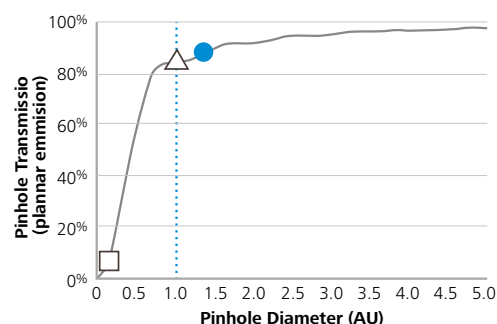
## Evolution to Airyscan 2

The original 2014 Airyscan detection concept from ZEISS features a 32-channel gallium arsenide phosphide photo-multiplier tube (GaAsP-PMT) area detector that collects a pinhole-plane image at every scan position. Each detector

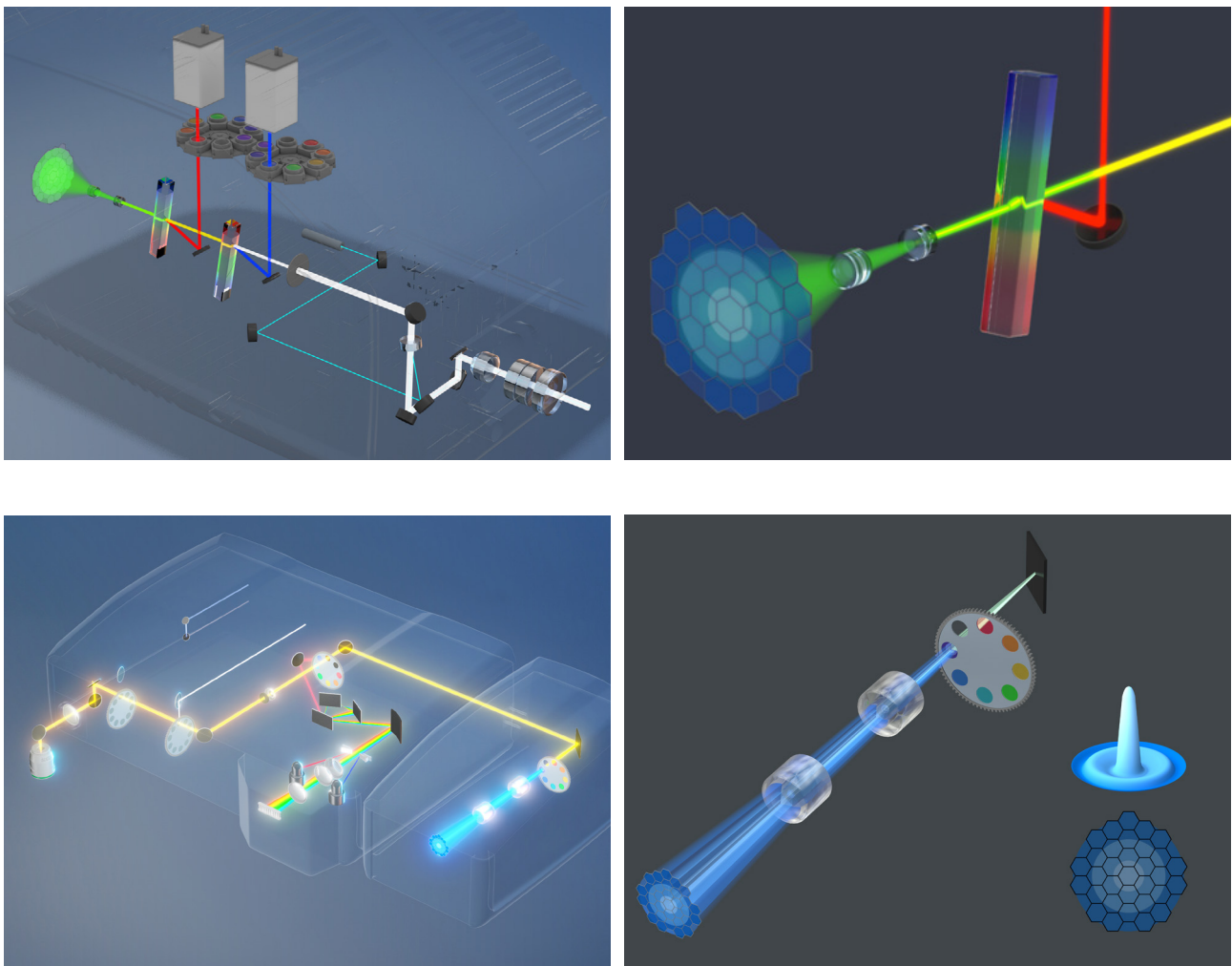
element functions as a single, very small pinhole where the data from each element carries not only intensity information but light distribution and location information. This approach is advantageous as a 1.4 increase in optical resolution is provided by each detector element with the total light collection of 1.25 airy units (AU). Once a the final image is calculated from the 32 elements a simultaneous increase of 4-8x in signal-to-noise as well as two fold spatial resolution improvement over widefield imaging is provided [Figures 1 and 2].<sup>[4-8]</sup> Hence the application of the original Airyscan concept on the ZEISS LSM 8 series provided researchers the ability to study structures and dynamics and processes previously not possible.<sup>[1-3]</sup>



- Traditional Confocal @ 0.2 AU
- △ Traditional Confocal @ 1 AU
- Airyscan



**Figure 2** Plots showing the lateral resolution, optical slice thickness and collection efficiency of a traditional confocal compared to Airyscan 2.

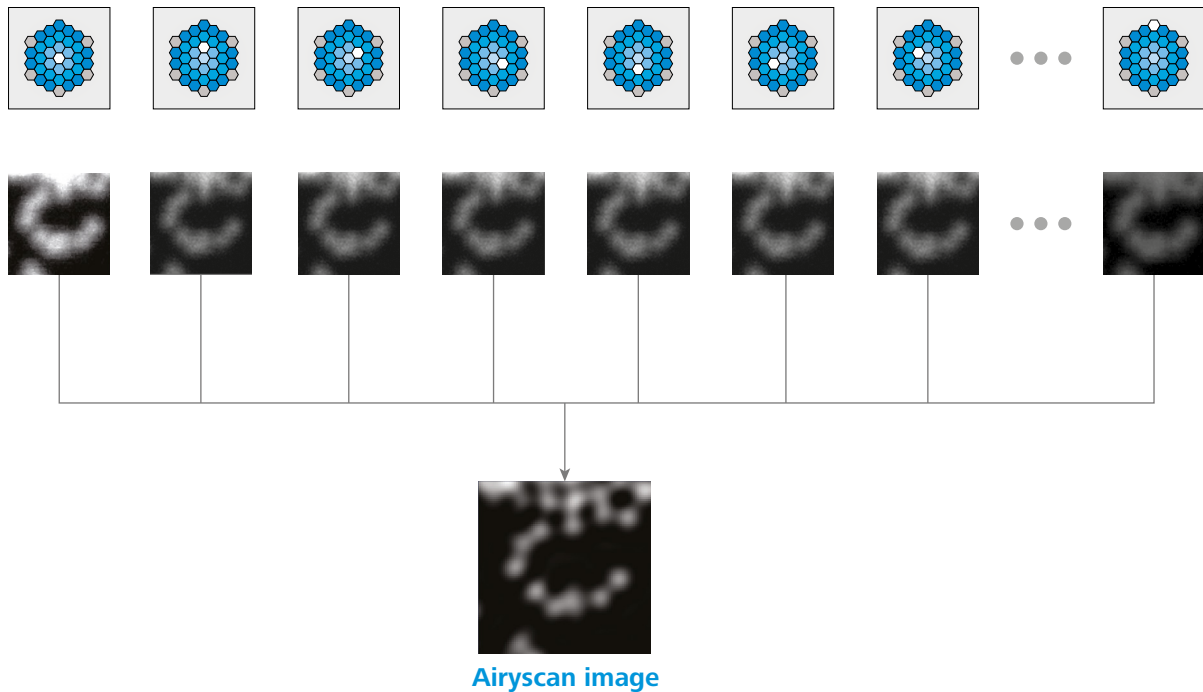


**Figure 3** (Top row) Beampath schematic of LSM 900 with Airyscan 2 zoom up. (Bottom row) Beampath schematic of LSM 980 with Airyscan 2 zoom up.

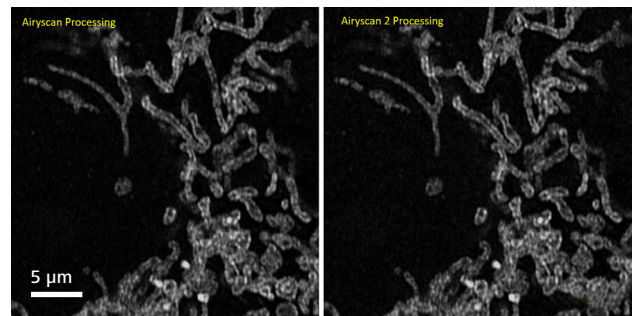
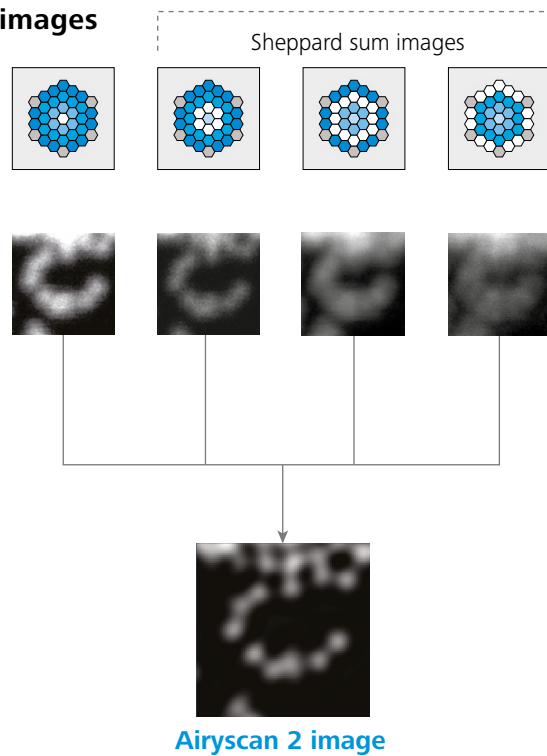
The evolution to Airyscan 2 focuses on streamlining how image data are handled thereby reducing data size and improving reconstruction times. Like the original Airyscan concept, the backbone of the new Airyscan 2 detector from ZEISS features a 32-channel GaAsP-PMT area detector that collects a pinhole-plane image at every scan position [Figure 3]. However, instead of carrying 32 elements of information per pixel, improved data handling schemes allow a new preprocessing step where the information from the 32 elements is transformed into 4 “rings” of information per pixel [Figure 4]. The change in data structure affords researchers a 6.6x decrease in raw data size improving data handling times and resources (copying/saving/storage),

but also greatly improving the time for image reconstruction by a factor of 5x [Table 1]. As shown in Figure 3 the new data handling schemes maintain the same SNR improvements and superresolution capabilities of the original Airyscan concept. In addition to innovative data handling schemes of Airyscan 2, fully leveraged realtime electronic schemes have enabled allowing substantial increase in scanning speeds by increase the data transmission rate for Airyscan 2 on the LSM 900 and LSM 980. Combined with the increased scanning speeds of the Airyscan 2 system, the biggest jump in performance of Airyscan 2 resides with the new innovative Multiplex mode for Airyscan 2.

## 32 images



## 4 images



**Figure 4** (Top) Initial data handling for Airyscan carried 32 images per pixel which were utilized to reconstitute a final image with superresolution and a 4-8x SNR improvement. (Left) New data handling schematic for Airyscan 2 where 4 'rings' of information are kept in place of the original 32 elements. The new scheme enables 6.6x smaller data sizes and ~5x faster image reconstitution. (Above) Image example of TOMM20 labeled cells comparing data handling from Airyscan (left) and Airyscan 2 (right). The Airyscan 2 data handling scheme provides the resolution and SNR increase without sacrificing image quality.

	Airyscan	Airyscan 2
<b>Resolution (Increase)</b>	120nm/350nm (2x increase)	120nm/350nm (2x increase)
<b>SNR Increase</b>	4x-8x	4x-8x
<b>Data Size (1024x; 1024y; 150Z)</b>	40.6 GB	6.2 GB
<b>Processing Time (1024x; 1024y; 150Z)</b>	1 minute 25 seconds	17 seconds

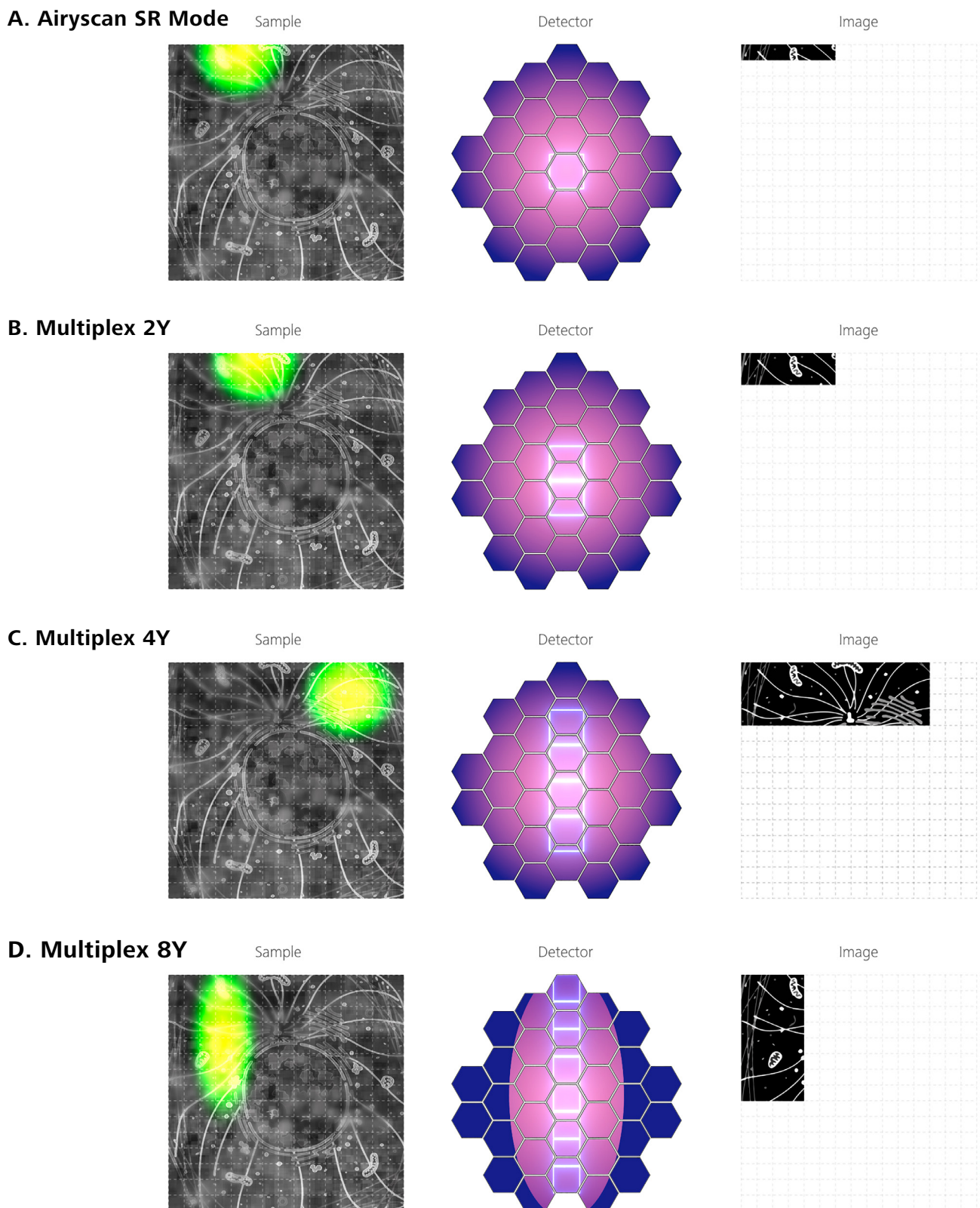
**Table 1** File size and processing speed comparison between Airyscan and Airyscan 2. The data handling scheme of Airyscan 2 enables a 6.6x reduction in file size with a 5x increase in processing time enabling greater usability and throughput.

### The New Multiplex Mode

The utilization of a physical aperture (i.e. pinhole) and unitary detector (standard PMT) in the design of traditional laser scanning microscopy systems require that the final data resolution, SNR and speed to be directly coupled to how finely image is scanned and how the data is read off the PMT. The traditional design yields a 1:1 relationship between the scanner movement to the data read out where for every scan position (i.e. pixel) there is one value read from the unitary detector (i.e. pixel intensity). Hence, the scanner-detector relationship directly impacts what model systems, experiments or applications can be performed on a laser scanning system as researchers have traditionally

been forced to compromise between scanning speed (i.e. frame rate), pixel dwell time (i.e. signal to noise and laser exposure) or pixel size (image resolution). The interplay and co-dependence that was once leveraged as experimental flexibility has now become the limiting factor for traditional confocal systems. With model systems and organisms get larger with lower expression levels (i.e. lower fluorophore concentrations) traditional confocal systems are severely restricted to how quickly desired sample volumes can be scanned with sufficient SNR and resolution ultimately impacting the ability to quantify the image data.

The innovative design of the ZEISS Airyscan 2 detector enables the expansion of the scanner/detector co-dependence beyond the traditional 1:1 relationship by fully leveraging the positional information contained in the pinhole plane. The power behind the new Multiplex mode is that both the structure of the excitation laser and the structure of the detection are leveraged to parallelize image acquisition while still providing simultaneous higher signal-to-noise and superresolution [Figure 5]. The Airyscan 2 detector always captures a detailed image of the pinhole plane allowing the excitation laser to be stepped farther/coarser over the field of view to improve acquisition speed. Due to the high amount of spatial information captured in the pinhole plane image, a final image is reconstituted with finer resolution than the acquisition sampling. Therefore, with Multiplex mode for Airyscan 2 the final image resolution is dictated by the detection sampling and not the excitation sampling. By changing the acquisition sampling steps to match the inherent oversampling of the pinhole plan imaging, image acquisition can be parallelized from 2x up to 8x fold while still providing superresolution imaging with superior SNR at speed (Figure 5).



**Figure 5** Depictions of the image acquisition schemes available on the new Airyscan 2. (A) Standard Airyscan SR imaging. (B) Available on the LSM 900 the Multiplex 2Y mode acquires two image pixels in parallel. (C) Available on both the LSM 900 and LSM 980 the Multiplex 4Y mode acquires four image pixels in parallel. (D) Available on the LSM 980 the Multiplex 8Y mode acquires 8 image pixels in parallel.

## LSM 900

Mode	Confocal	Airyscan SR	Multiplex SR-2Y	Multiplex SR-4Y	Multiplex CO-2Y
Parallelization	1	1	2	4	2
Max. resolution X/Y nm	—	120/120	140/140	140/140	Confoc. or better
Max. resolution Z nm	—	350	450	450	Confoc. or better
Max. FPS @ 512x512	4.7	4.0	8.4	18.9	8.3
FPS @ max FOV (mm)	0.5 (@Zoom 0.5 / SF18)	0.4 (@Zoom 1.3 / SF7)	0.8 (@Zoom 1.3 / SF7)	3.5 (@Zoom 1.3 / SF7)	3.5 (@Zoom 1.3 / SF7)
Processing 1kx1k /150 slices	—	< 30s	< 30s	< 30s	<30s
SNR vs convent. confocal	—	4-8x	4-8x + speed	4-8x + speed	—

## LSM 980

Mode	Confocal	Airyscan SR	Multiplex SR-4Y	Multiplex SR-8Y	Multiplex CO-8Y
Parallelization	1	1	4	8	8
Max. resolution X/Y nm	—	120/120	140/140	120/160	Confoc. or better
Max. resolution Z nm	—	350	450	450	Confoc. or better
Max. FPS @ 512x512	6.1	4.7	25.0	47.5	34.4
FPS @ max FOV (mm)	0.4 (@Zoom 0.6 / SF20)	0.2 (@Zoom 1.7 / SF7)	1.0 (@Zoom 1 / SF12)	2.0 (@Zoom 1 / SF12)	9.6 (@Zoom 1 / SF12)
Processing 1kx1k /150 slices	—	< 30s	< 30s	< 30s	< 30s
SNR vs convent. confocal	—	4-8x	4-8x + speed	4x + speed	—

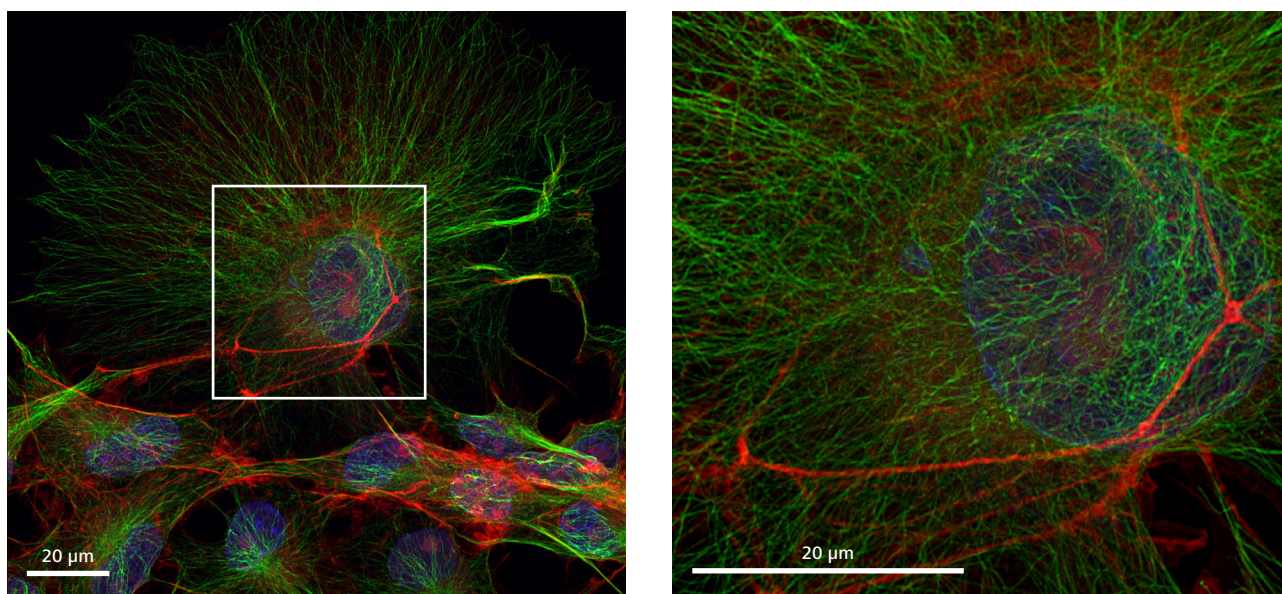
**Table 2** Overview of Multiplex mode for Airyscan 2 performance for both LSM 900 and LSM 980. All values determined for the objective 63x/1.4 and the laser wavelength 488nm. Max. resolution X/Y measured with Nanoruler samples for SR modes. Maximal Speeds are measured at variable zoom required for preferred sampling with selected ROI.

The Multiplex mode for Airyscan 2 is available on both the LSM 900 and LSM 980 offering the ability generate confocal (CO) or superresolution images (SR). The CO-2Y/SR-2Y and SR-4Y acquisition strategies are available on either the LSM 900 (2Y & 4Y) and LSM 980 (4Y) providing parallelization factors of either 2x or 4x while keeping a 4x improvement in SNR (over traditional confocal) with a choice of confocal resolution or superresolution (Table 2). Using the SR-4Y acquisition strategy, simultaneous improvements in resolution, SNR and imaging speeds enable larger fields of view and/or higher volumetric imaging rates with better image quality than traditional confocal microscopy (Figure 6).

For maximum volume acquisition rates, the Multiplex mode for Airyscan 2 on the LSM 980 needs to be utilized.

Like the Fast mode for Airyscan on the LSM 880, Multiplex mode for Airyscan 2 on the LSM 980 utilizes excitation shaping approach in combination with pinhole plane imaging.<sup>[5]</sup> By slightly stretching the excitation beam along the y-dimension the detection sampling approach of the Multiplex mode for Airyscan 2 is employed to provide an 8x parallelization in acquisition. Hence, the CO-8Y/SR-8Y utilizes very coarse acquisition stepping of the excitation laser providing 8x improvements in imaging speeds (over traditional confocal) while also maintaining a 4x increase in SNR with a choice of confocal resolution or superresolution. The increase in acquisition speed can be leveraged in single planes for ultrafast time series, rapid tiling of large areas or fast volumetric time lapses [Figure 6].





**Figure 6** Comparison of the field of view you can image at superresolution in the same time using the Airyscan SR (right) and Multiplex mode (left). COS 7 cells with labelled microtubules (alpha-tubulin 488, green) and actin (phalloidin 647, red).

[https://p.widencdn.net/lzdcrc/Cilia-moving-04.czi\\_Super-Resolution](https://p.widencdn.net/lzdcrc/Cilia-moving-04.czi_Super-Resolution)

Live imaging with 143 frames per second of fluo-rescently labeled motile cilia of brain ependyma. Acquired with Airyscan CO-8Y mode combining image quality and speed; for detailed analysis of ciliary beating direction and frequency.

Courtesy of G. Eichele, Department of Genes and Behavior, Max Planck Institute for biophysical Chemistry, Göttingen, Germany.

<https://p.widencdn.net/a5c2vp/New-15-3D-Rendering-Time-and-Depth-projection>

Meiosis in starfish oocytes. The rendering is a projection of the process along z-axis (maximum intensity) and time (color-coded projection); to illustrate the movement of the chromosomes within the volume of the nucleus. The movie shows the transport of chromosomes, labeled by Histone 1-Alexa 568, in a starfish oocyte undergoing meiosis. A z-stack of 67  $\mu\text{m}$  was acquired every 2.4 seconds with Airyscan CO-8Y mode. Concomitant with chromosome transport, the nucleolus (the large spherical structure) is disassembling.

Courtesy of P. Lenart, MPI for Biophysical Chemistry, Göttingen, Germany

## Conclusion

Focused on streamlining data handling and decreasing image reconstruction times, the evolution of Airyscan 2 provides new data handling concepts providing 6.6x smaller data sizes and 5x faster image reconstruction times. Further, optimized real time acquisition strategies employed with the LSM 9 series enable faster scan speeds for the Airyscan 2 allowing improved data throughput. The new Multiplex mode for Airyscan 2 innovative imaging strategy allows to address the current development in biomedical research

to not only capture less signal and provide statistical sound data. With a focus on extending Airyscan imaging to larger model systems with lower expression levels, the Multiplex mode concept increases acquisition speeds even further while simultaneously providing superresolution capabilities and a 4x SNR improvement over traditional confocal. The novel detection sampling concept allows rapid volumetric imaging with unprecedented resolution beyond what is available in traditional confocal systems today.

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