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The new 2D Superresolution mode for ZEISS Airyscan

Utilizing a pinhole-plane imaging concept, Airyscan allows for simultaneous improvement in resolution and signal-to-noise by capitalizing on an innovative 32-channel GaAsP photomultiplier tube (PMT) array detector. Each detection channel functions as a very small pinhole to increase resolution while the overall detector design delivers better signal-to-noise than traditional GaAsP-based confocal systems. In the past, a stack of at least five *z*-slices had to be deconvolved to get usable images with an optical section thinner than 1 Airy unit. Now, the new 2D Superresolution mode for Airyscan delivers images with the thinnest optical section (0.2 Airy units) from a single image while maintaining the light collection efficiency of a much larger 1.25-Airy-unit pinhole.

The creation of optical section images in biomedical research started in 1955, when Marvin Minsky laid the foundation for confocal microscopy[1]. In an effort to improve the contrast of images for the study of the central nervous system, Minsky placed an aperture in a conjugate plane relative to the microscope objective. He discovered that he could create an optical section within a three-dimensional biological sample by blocking any light that did not originate from the focal plane. Hence, Minsky was able to generate high-contrast images to study nervous system circuitry (prepared via the Golgi method) free from out-of-focus scatter from imaging planes deep within scattering samples. This confocal concept was the foundation of modern laser scanning confocal microscopy.

Creation of the optical section

Conventional confocal laser scanning microscopes leverage a combination of a physical aperture (the so-called pinhole) and a unitary detector (PMT, hybrid detector, avalanche photodiode, etc.) to create an optically sectioned image[2]. Naturally, by varying the aperture size, one can also change the thickness of the optical section (**Fig. 1a**). In addition to the thickness of the optical section, the resulting image resolution and image signal-to-noise are directly related to the aperture size (**Fig. 1b**,**c**). As the pinhole is closed, less signal can reach the unitary detector, which significantly reduces the image signal-to-noise. Traditional confocal microscopes demand the sacrifice of optical sectioning and resolution for the sake of a usable signal-to-noise ratio and acceptable sample bleaching and phototoxicity. Consequently, a pinhole size of 1 Airy unit (AU) has become the default for confocal microscopes.

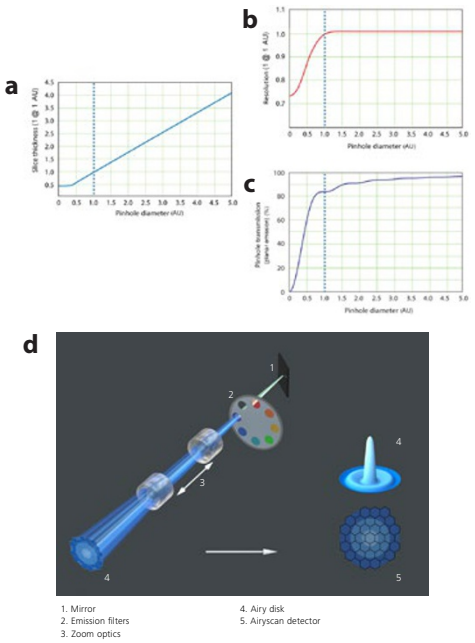

Figure 1 | By projecting 1.25 AU onto a 32-element GaAsP PMT array, each element of the detector represents a separate 0.2-AU pinhole. This arrangement allows Airyscan to simulatanously improve confocal resolution and increase image signal-to-noise. (**a**–**c**) The dependence of optical section thickness (**a**), resolution (**b**) and signal transmission (**c**) on pinhole diameter. (**d**) An illustration of the Airyscan principle, showing the projection of 1.25 AU onto a 32-element GaAsP PMT array.

In contrast to traditional confocal detectors, Airyscan combines the resolution benefits of imaging with a small pinhole with the collection efficiency of a large pinhole. This design delivers a simultaneous increase in resolution and signal-to-noise by projecting 1.25 AU onto a 32-channel GaAsP PMT array detector. Each detector element behaves as a 0.2-AU pinhole while the collection efficiency of a 1.25-AU pinhole is maintained[3,4] (**Fig. 1d**). Moreover, linear deconvolution is used to extend the resolution beyond what a 0.2-AU pinhole provides,

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resulting in a resolution increase in all three spatial dimensions. The new 2D Superresolution mode for Airyscan improves the optical sectioning by a factor of 2.4 for a single optical section by utilizing a new exclusive processing algorithm in place of the linear deconvolution step[5,6] (**Fig. 1a**). Along with improved optical sectioning, Airyscan now provides lateral resolution to 120 nm for 2D and 3D data sets (*z*-stacks) and 350-nm axial resolution for *z*-stacks (**Fig. 2**).

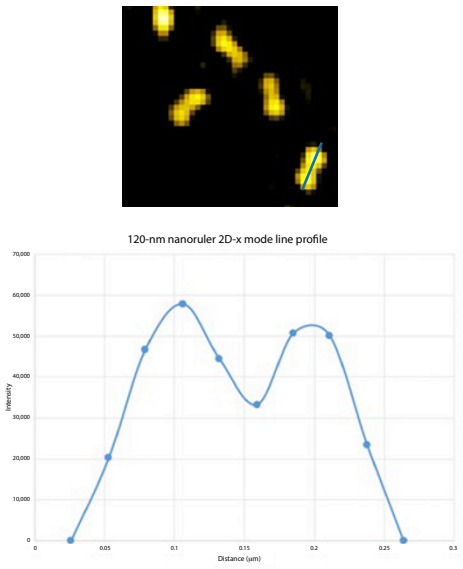

Figure 2 | Airyscan technology can resolve the labeled ends of nanorulers with a defined distance of 120 nm, using a 2D image (one optical section). The blue measurement line in the image at the top represents a distance of 110 nm, and both ends can be clearly discriminated. The nanorulers used for this image were GATTA-SIM 120B (distance, 120 nm; Alexa Fluor 488) from GATTAquant DNA Nanotechnologies, www.qattaquant.com.

The new 2D Superresolution mode

Since its launch, Airyscan's purpose has been to ensure that all precious fluorescence emission photons are preserved to provide the highest sensitivity of any confocal. This focus on system sensitivity has resulted in unprecedented image quality, resolution and sectioning for 3D stacks. Airyscan 2D images have always offered an improvement in lateral resolution while maintaining an optical slice thickness of 1.25 AU (**Figs. 3** and **4**).

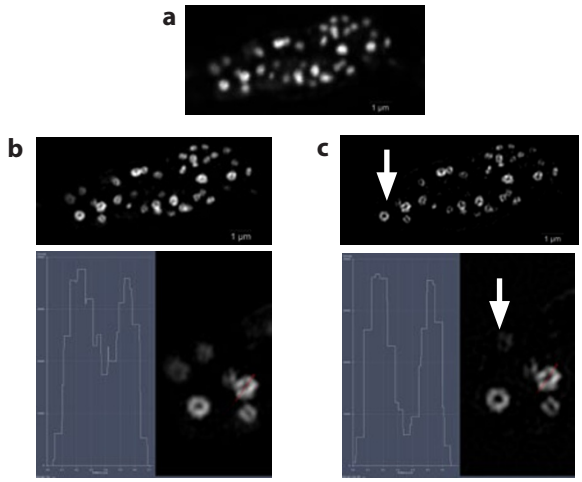

Figure 3 | *Drosophila melanogaster* neuromuscular junctions stained for Bruchpilot (BRP). (**a**) BRP is arranged in a characteristic ring-like structure, which cannot be resolved by confocal imaging. (**b**,**c**) Airyscan 2D processing is able to resolve most of the structure (**b**), and the new Airyscan 2D Superresolution mode reveals even more detail (**c**). Some gap junctions that are slightly visible but out of focus in **b** are no longer present in **c** (arrows) as a result of the thinner optical section. Sample courtesy of J. Pielage, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.

The new 2D Superresolution mode for Airyscan takes advantage of the fact that the confocal point spread function entangles information from the *x*, *y* and *z* planes in the pinhole plane. Airyscan measures the emission fluorescence distribution already in a single image acquisition, thereby yielding information about how the signal is entangled. The new exclusive algorithm of the 2D Superresolution mode makes it possible to distinguish and separate the light originating in the focal plane from light originating outside of the focal plane. This

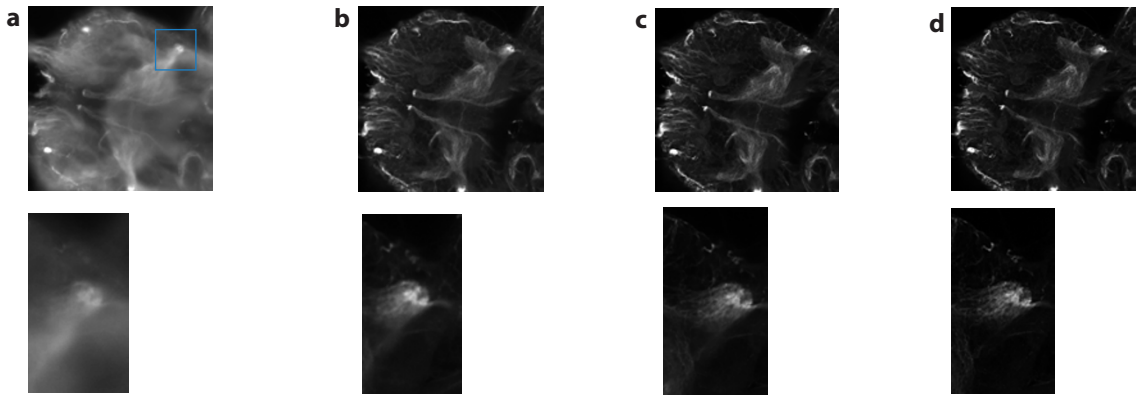

Figure 4 | This series of images, showing single sections of the anterior central nervous system (labeled with Alexa Fluor 561) of a larval *Platynereis dumerilii*, depict the increasing sectioning power of confocal imaging techniques. (**a**–**d**) In fluorescent widefield (**a**), most of the structural information is lost because of the out-of-focus light contributing to the image. To eliminate this blur, confocal microscopes remove the out-of-focus emission (**b**) by producing optical sectioning via a pinhole. Capturing the same image section with an Airyscan detector improves the resolution of finer structures even further (**c**) and finally produces a perfect optical section in 2D Superresolution mode (**d**) by using the best resolution of a single optical slice. The blue square in the upper image in **a** highlights the region shown at higher magnification below; this same region is highlighted in **b**–**d**.

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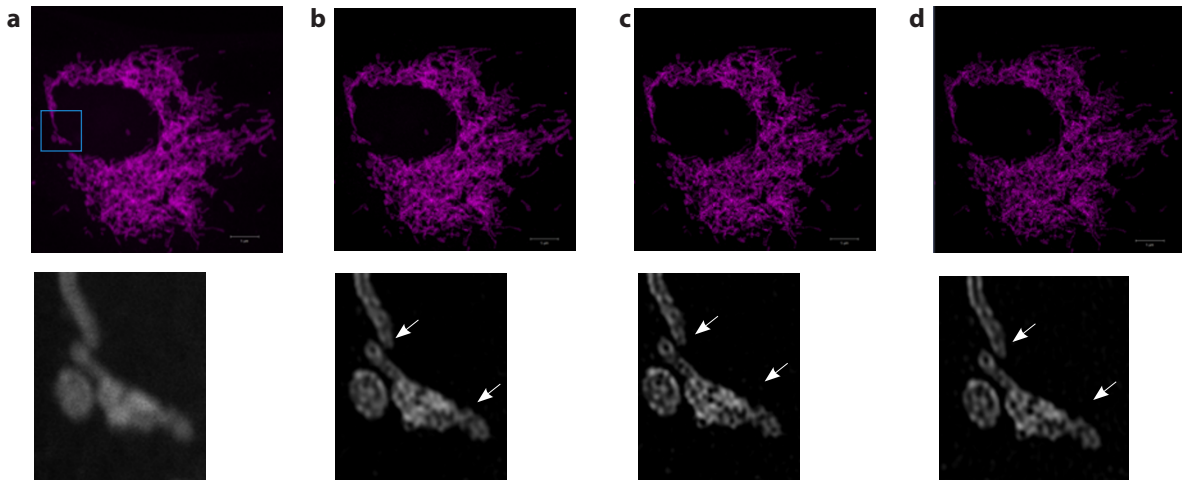

Figure 5 | TOMM20, a protein located on the outer membrane of mitochondria, is labeled by Alexa Fluor 568 in this series of images depicting single optical slices. (**a**,**b**) Although the lumen of the mitochondria cannot be resolved by pure confocal imaging (**a**), it can be visualized by Airyscan imaging and 2D processing (**b**). (**c**) To improve the resolution even further with Airyscan, the acquisition of several *z*-slices was necessary (one optical section is depicted). (**d**) The full resolution potential can now be utilized with a single *z*-section, limiting light exposure and time to the absolute minimum. Note the differences between Airyscan 2D processing (**b**), Airyscan 3D processing (**c**) and 2D Superresolution mode processing (**d**) (arrows), with clear visualization of the mitochondrial lumen in **c** and **d**. The blue square in the upper image in **a** highlights the region shown at higher magnification below; this same region is highlighted in **b**–**d**. Sample courtesy of M. Davidson, The Florida State University, Tallahassee, Florida, USA.

allows researchers to obtain perfect optical sections, without the need to acquire a z-stack (**Figs. 3**–**5**). To get the same optical section on a traditional confocal, a researcher would have to close the pinhole to 0.2 AU and sacrifice >95% of all emission signal (in both the plane of interest and out-of-focus planes). This would limit the information that could be obtained from the plane of interest.

Summary

The Airyscan detector from ZEISS delivers more information from the pinhole plane than traditional confocal microscopes. With this information, researchers get simultaneous improvements in resolution and signal-to-noise and an increase in acquisition speeds. The new 2D Superresolution mode improves the achievable resolution of a confocal microscope to 120 nm laterally and delivers a 2.4× thinner optical section. In image stacks, an axial resolution of 350 nm is achieved.

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