



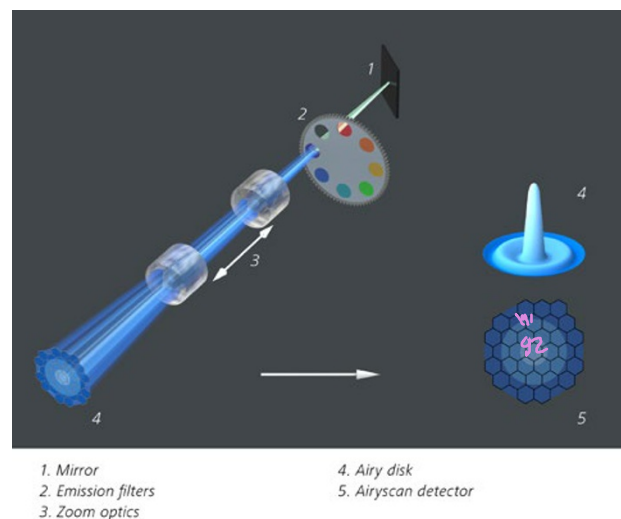
## The Airyscan detector from ZEISS: confocal imaging with improved signal-to-noise ratio and super-resolution

With Airyscan, ZEISS introduced a new detector concept for confocal laser-scanning microscopy (LSM). Whereas traditional LSM designs use a combination of pinhole and single-point detectors, Airyscan is a 32-channel gallium arsenide phosphide photomultiplier 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. Knowledge about the beam path and the spatial distribution of each detector channel enables very light-efficient imaging with improved resolution and signal-to-noise ratio.

Over the past 25 years, confocal imaging has become the standard technique for most fluorescence microscopy applications. The increased use of confocal imaging systems in basic biomedical research can be attributed to their ability to produce high-contrast, optically sectioned images while providing enough acquisition versatility to address many sample and application demands<sup>1</sup>. The manufacturers of most commercially available confocal imaging systems have, over the past 25 years, developed new approaches and options to increase image contrast and instrument versatility. However, what has not changed is the fundamental aspect of a confocal imaging system: the creation of the optical section.

Traditionally, the optical-sectioning ability of a confocal imaging system is the result of a field stop, the so-called pinhole, placed in a conjugate image plane in front of a detector along the fluorescence detection path. If the pinhole is sufficiently closed, out-of-focus light collected by the objective will be blocked from the detector, creating an optically sectioned image. Hence the hallmark of every commercial LSM system has been the use of a physical aperture for a pinhole in combination with a unitary detector (typically a PMT). However, in the Airyscan detector from ZEISS, the traditional pinhole-and-detector design has been reworked to offer greatly improved resolution and signal-to-noise ratio (SNR). Introduced in 2014, the Airyscan detector contains a hexagonally packed detector array instead of a physical confocal pinhole aperture and unitary detector (**Fig. 1**). Just like detectors with the traditional confocal pinhole, the Airyscan detector is positioned in a conjugate focal plane relative to the excitation spot and uses zoom optics to project a defined number of Airy unit (AU) orders onto the detector. Because it collects the additional information of a pinhole-plane image at every

excitation scan position, the Airyscan detector offers substantial and immediate benefits over traditional confocal microscopy systems by increasing both the spatial resolution and the SNR of all images while maintaining the optical-sectioning ability of a traditional confocal microscope<sup>2</sup> (**Fig. 1**).



**Figure 1** | The Airyscan principle.

### Increased spatial resolution

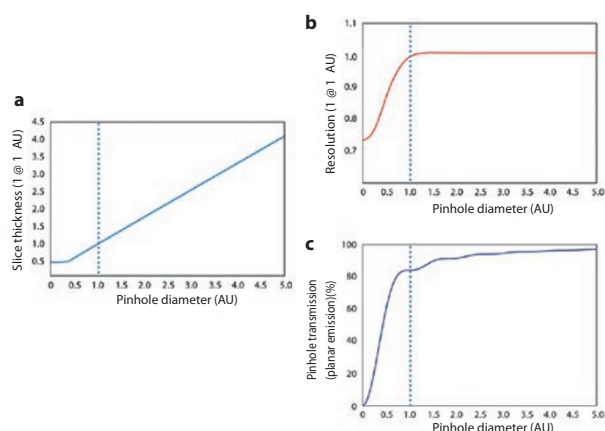
The spatial resolution of a traditional laser-scanning microscope is a product of the illumination point-spread function and the detection point-spread function, and the size of the pinhole opening has a considerable effect on the spatial resolution of the resulting image (**Fig. 2**). In theory, the maximum resolution of a traditional confocal imaging system would be achieved with a pinhole diameter of 0.2 AU, which should result in an  $\sim 1.4\times$  increase in spatial resolution

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## APPLICATION NOTES

compared with that obtained with the traditional 1-AU pinhole. However, the downside to using a pinhole with a diameter less than 1 AU is the dramatic decrease in signal reaching the detector (95% loss at 0.2 AU). If the signal reaching the detector is decreased, the resulting image quality will also decrease. This presents an obstacle to the use of smaller pinhole diameters in most biomedical imaging applications, as most biological samples and fluorophores cannot supply enough fluorescence (because of photodamage and/or phototoxicity) to yield images with sufficient SNR. As a result, the pinhole is traditionally set to a size equivalent to 1 AU to offer the best compromise between optical sectioning and SNR.



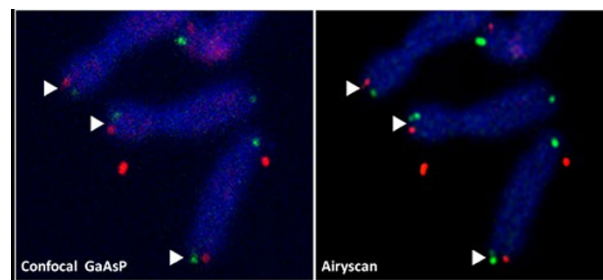
**Figure 2** | Characterization of the effect of pinhole size on resolution and SNR in confocal microscopy. As shown, the smaller the pinhole, the smaller the optical section and higher the resolution. However, the smaller the pinhole, the worse the image SNR. Traditionally, a pinhole size of 1 AU is used to offer the best compromise between resolution and image SNR. **(a)** Effect of pinhole diameter on optical-slice thickness. **(b)** Lateral resolution as a function of pinhole diameter. **(c)** Effect of pinhole diameter on transmission efficiency.

In contrast to a traditional laser-scanning microscope, the novel detector design of Airyscan combines the resolution benefits of imaging with a small pinhole with the collection efficiency of a large pinhole (**Fig. 1**). Airyscan achieves both of these attributes by projecting 1.25 AU onto the detector (via zoom optics), where each detector element behaves as a small, 0.2-AU pinhole, while the collection efficiency of a 1.25-AU pinhole is maintained. Moreover, because only 1.25 AU is projected onto the Airyscan detector, the optical-sectioning ability of LSM is also maintained. To extend the resolution beyond what a 0.2-AU pinhole provides, Airyscan uses a linear deconvolution, resulting in a 1.7× increase in resolution in all three spatial dimensions (140 nm in x and y, and 400 nm in z) (**Fig. 3**).

## Increased SNR

The improvement in SNR achieved with the Airyscan detector is directly related to two effects. First, the larger overall size of 1.25 AU allows the pinholes to collect up to 50% more light than a conventional 1-AU pinhole (**Fig. 1**). Second, the small individual pinholes not only extend the resolution beyond a conventional light-scanning microscope's resolution limit, but also raise the

contrast of higher spatial frequencies collected by the confocal microscope system<sup>3,4</sup>. In other words, the Airyscan detector gives improved contrast without increasing noise, which directly translates to a substantial (4–8×) increase in SNR in the final image (**Fig. 3**). Importantly, in order for a traditional confocal system to have the same SNR, a compromise in speed, resolution or sensitivity (or a combination of those) would have to be made.



**Figure 3** | Telomere replication without RTEL1. Stalled forks and telomere breakage are visualized as doubled dots using Airyscan (white arrowheads). Resolution is meaningless without good SNR. Image courtesy of J. Karlseder (Molecular and Cell Biology Laboratory) and J. Fitzpatrick (Director, Waitt Advanced Biophotonics Core), the Salk Institute, La Jolla, California, USA.

## Summary

The novel detector design of Airyscan overcomes limitations of the classical assembly consisting of a physical pinhole and a unitary detector and uses a new pinhole-plane image-detection approach based on a 32-channel GaAsP-PMT area detector. In Airyscan, each of the 32 detector elements acts as its own small pinhole with positional information. The new positional information allows for increased contrast of high-spatial-frequency information previously not available in traditional confocal systems. The increase in spatial-frequency contrast enables Airyscan to produce images with substantially increased SNR and resolution compared to LSM images acquired with a 1-AU pinhole. Ultimately, Airyscan delivers 1.7× higher resolution in all three spatial dimensions and increases the SNR by 4–8× compared with traditional LSM systems with a 1-AU pinhole.

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