

Upgrade of a facility confocal microscope with an Abberior stimulated emission depletion (STED) microscopy module

In regular optical microscopy, resolution is subject to a diffraction limit: Due to the wave nature of light, the signal from a point like source is smeared out into a 3D point spread function or, considering exclusively the focal plane, a 2D Airy disc (Figure 1). This limits the minimum distance at which two pointlike light sources can still be distinguished. Under ideal circumstances this corresponds to a distance $D_{\text{diff}} = 0.61 \lambda / \text{NA}$, with λ the wavelength of the light and NA the numerical aperture of the objective. Critically, this diffraction limit also operates for excitation, enforcing a minimum size for the diameter of the excitation spot of a point scanning confocal microscope.

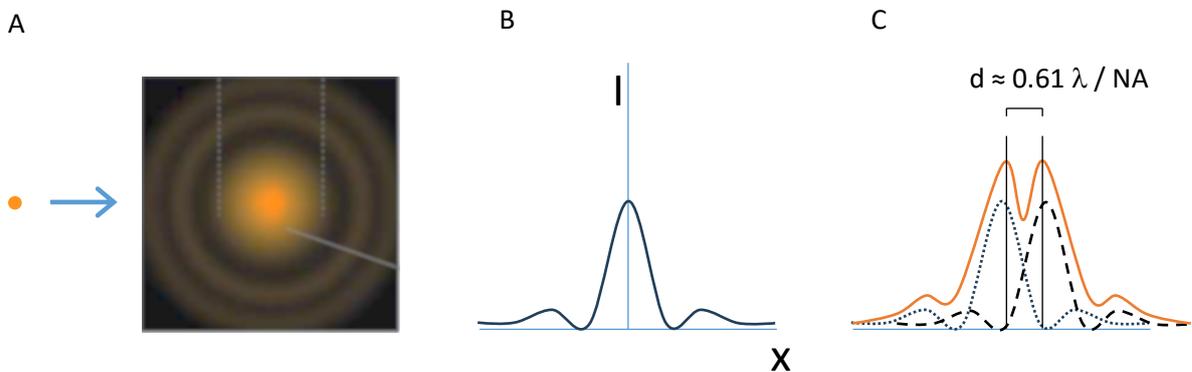


Figure 1: Diffraction limits the resolution of conventional microscopy. A,B) Diffraction blurs the light emanating from a point source into an Airy disc (A) with a central intensity maximum (I) and weaker secondary peaks (B). C) According to Rayleigh's criterium the diffraction limit can be defined as the distance between two point sources (solid and dashed black lines) where the maximum of the first falls into the first minimum of the second, resulting in a dip between the peaks of the combined signal (orange).

For real, biological samples, even using high NA objectives, the practically achievable resolution of confocal microscopy is of the order of half the excitation wavelength, i.e. around 250 nm for standard visible light lasers. However, many interesting biological structures or processes occur at scales of tens rather than hundreds of nm. To resolve these by direct imaging thus requires microscopic **methods able to circumvent the diffraction limit**, that is, **superresolution imaging**.

STED microscopy works by first exciting a larger, diffraction-limited spot, which is then reduced to a sub-diffraction-limit excitation spot by a depletion laser, before scanning the object with correspondingly smaller pixel steps (Fig. 2).



Figure 2: The STED principle. Fluorescence is first excited in a diffraction-limited spot. A second laser pulse using a donut-shaped excitation volume depletes the excited state, turning fluorescence off everywhere except in the central hole. As the size of the donut hole is not diffraction limited, this leaves an excitation spot with sub-diffraction-limit diameter that can be used to scan the sample with superresolution.

In summer of 2025 we tested an Abberior STEDYCON STED superresolution microscope with resounding success.

The first sample was the nuclear pore complex, which due to its almost crystalline structure is an ideal molecular ruler to test the resolving power of superresolution microscopes: In each nuclear pore complex, 32 Nup96 molecules are organized in two stacked rings of 107 nm diameter, with each ring consisting of eight dimers evenly spaced around the circumference at 42 nm distance. Within each dimer, the Nup96 C-termini are spaced 12 nm apart (Fig. 3 A).

While in confocal microscopy individual nuclear pores appear as diffraction-limited spots (Fig. 3 B,C), a resolution of $< 100 \text{ nm}$ is required to resolve the ring-like structure, while a resolution of $< 40 \text{ nm}$ must be achieved to reveal the gaps between the dimers. We readily achieved this separation using our standard facility antibody staining protocol (Fig. 3 B,C), thus proving that the system does indeed reach the advertised superresolution of about 30 nm.

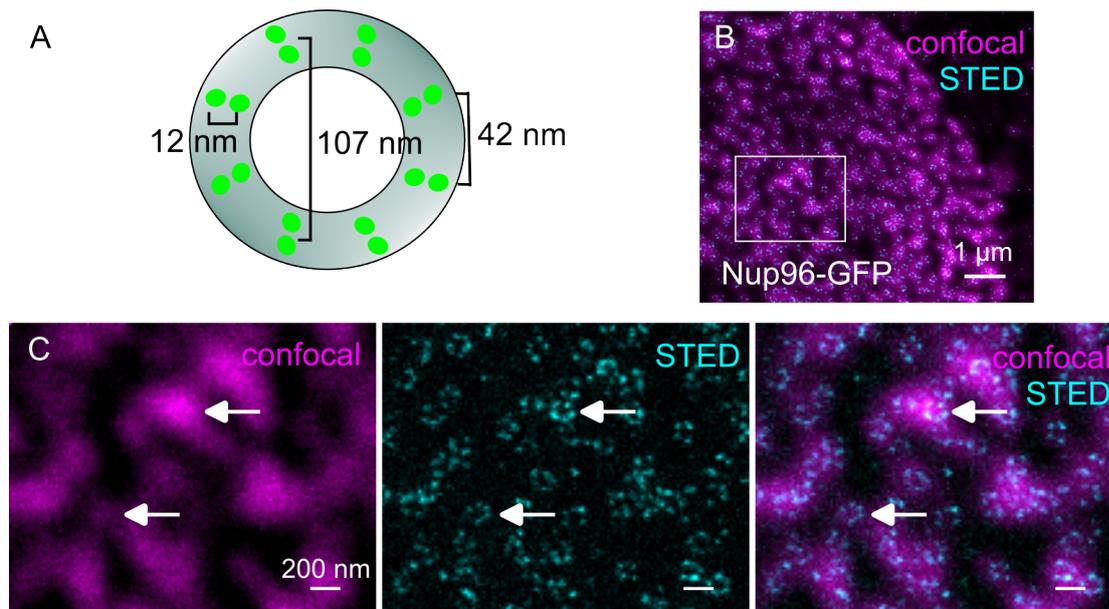


Figure 3: Testing the performance of the demo superresolution system using nuclear pore complexes as reference standards. A) Within a nuclear pore, 32 Nup96 subunits (green) are arranged in two stacked layers of 16 subunits each. Within each layer, the 16 subunits are distributed in a ring-like pattern of 8 dimers. Distances between Nup96 subunits are 12 nm within each dimer, 42 nm between adjacent dimers, and 107 nm across the ring. B) Anti-GFP immunostaining of the nucleus of a U2OS-Nup96GFP cell imaged in confocal (magenta) and STED mode (cyan). C) Magnification of boxed area in B). Nuclear pores appear as diffraction limited spots in the confocal image (magenta), while the STED image (cyan) both reveals the central gap and resolves the ring into distinct spots separated by dark pixels (arrows), indicating that a resolution of <40 nm was achieved. Note that not all Nup96-GFP subunits are labelled.

Similarly, we were able to further resolve diffraction-limited confocal images of desmosomes in alveolar epithelial cells (Fig. 4 A,B): Imaging the same field of view in STED mode revealed the gap between the intracellular desmoplakin C-termini on either side of the cell boundary (Fig. 4 C).

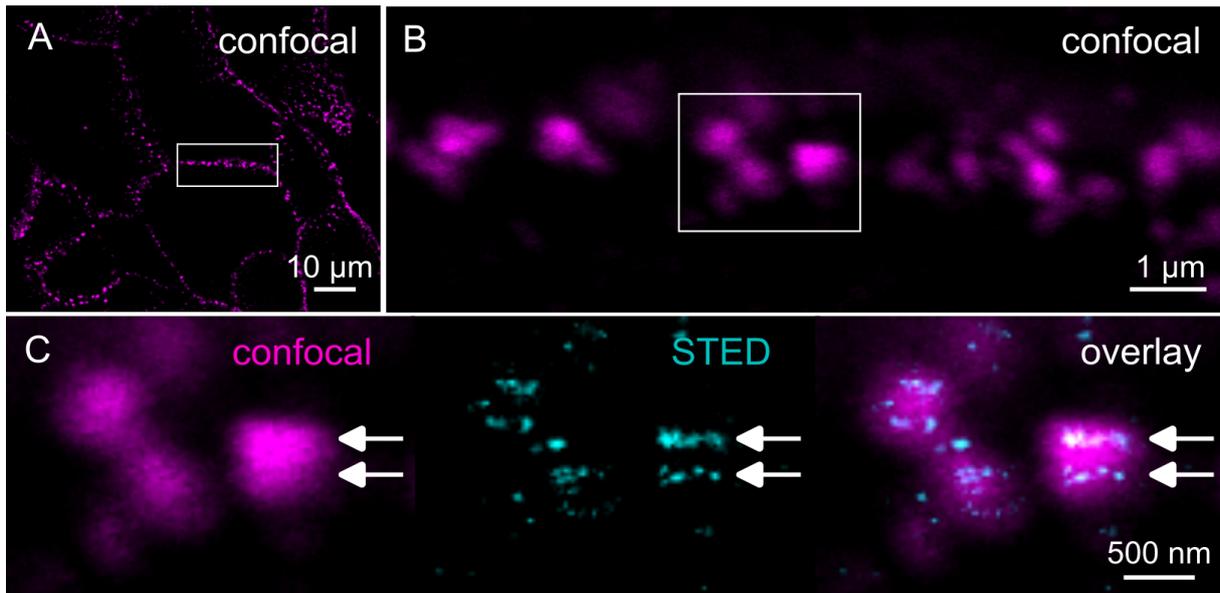


Figure 4: Resolving desmosomal subunits. A) Antibody staining against the intracellular C-terminus of Desmoplakin marks desmosomes at the cell boundaries of alveolar epithelial cells. In confocal images (A-C, magenta), desmosomes appear as diffraction-limited spots without discernible internal structure. In contrast, STED imaging of the same field of view (C, cyan) resolves distinct, non-contiguous desmoplakin clusters within each desmosome and reveals the gap (white arrows) between the desmoplakin C-termini on opposite sides of the cell boundary.

Importantly, STED microscopy also works with live cells, provided the target structures can be labelled using suitable dyes or transgenic red fluorescent proteins. We validated STED microscopy of mitochondrial substructure using the Abberior LIVE Orange mito dye. Again, STED is able to resolve suborganelle structures not accessible by diffraction-limited imaging, allowing e.g. the morphology-based assessment of mitochondrial maturation or health (Fig. 5).

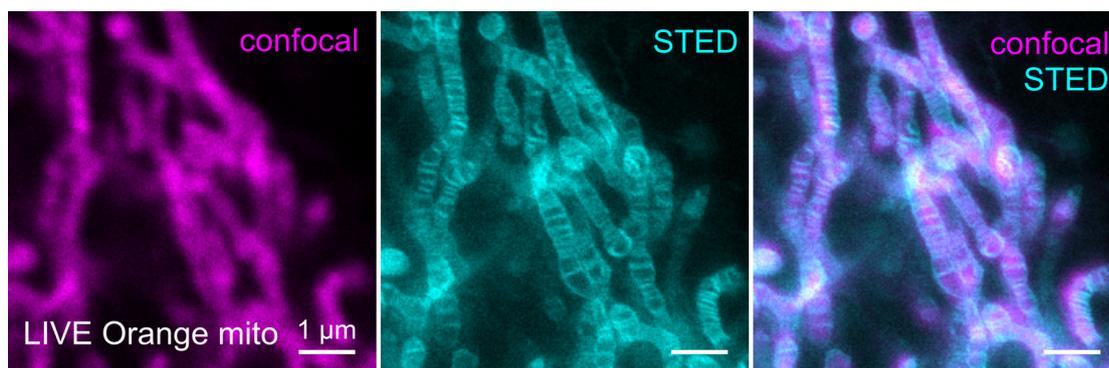


Figure 5: Resolving mitochondrial substructure in living cells. Mitochondria in live cells with the Abberior LIVE ORANGE mito dye appear in confocal images (magenta) as diffraction-limited objects with little discernible internal structure. STED imaging of the same field of view (cyan) reveals individual cristae, permitting e.g. morphology-based assessment of mitochondrial maturation. Shift between channels caused by the sequential imaging of moving organelles in live cells.

We therefore submitted a grant **application under the DFG 91B pilot program** for the upgrade and refurbishment of existing major research instrumentation, which was **approved in February 2026**.

The Abberior STEDYCON superresolution STED upgrade package for our existing Leica microscope will be delivered in the second quarter of 2026 and possess the following features:

- **Four independent colour channels** (DAPI to far red) with dedicated excitation lasers (405 nm, 488 nm, 561 nm, 640 nm) and detectors for operation in confocal mode.
- Pulsed excitation for use of the 561 nm and 640 nm channels to enable dual colour STED microscopy.
- A 775 nm depletion laser able to generate a depletion “donut” resulting in **30 nm resolution** at 100% depletion power.
- An active vibration isolation system for the base microscope unit.
- A dedicated, hardware autofocus system independent of the imaging laser.
- Highly sensitive detectors for all channels, operating in single photon counting mode for quantitative brightness measurements.
- Integration of the existing components such as the high precision XY stage and Z piezo actuator, climatization unit, and fluorescence illumination.
- Leica STED capable objectives for thinner (100x NA 1.4 oil immersion) and thicker samples (63 x NA 1.3 glycerol immersion with correction ring).