Maximize Insights from Your Sample

One detector, multiple possibilities

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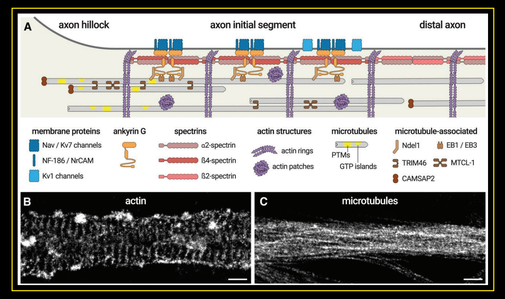
Resolution

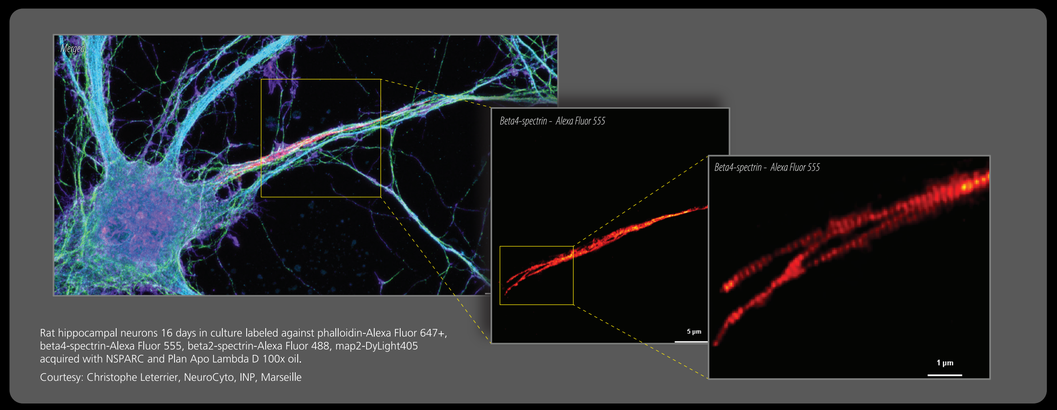
One remarkable discovery of super-resolution microscopy has been the periodic actin/spectrin scaffold along axons, with actin rings spaced every 190 nm by spectrin tetramers, crucial for structural support, maintaining axonal integrity, and regulating membrane processes such as endocytosis and exocytosis in neurons.

Techniques like STORM (Stochastic Optical Reconstruction Microscopy) or SIM (Structured Illumination Microscopy) can effortlessly resolve this pattern but require special preparation and are limited to surface imaging with dedicated objective lenses.

The Nikon Spatial ARray Confocal detector (NSPARC), based on image scanning microscopy, can unveil this scaffold and is not limited by sample thickness, objective lenses, or special sample preparation for improvement of signal to noise and resolution.



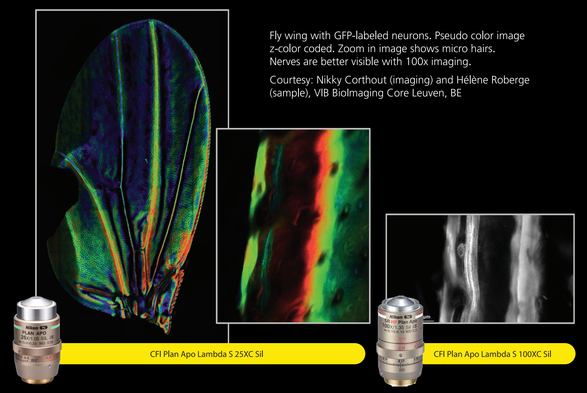
C. Leterrier, Journal of Neuroscience 28 February 2018, 38 (9) 2135-2145



Rat hippocampal neurons 16 days in culture labeled against phalloidin-Alexa Fluor 647+, beta4-spectrin-Alexa Fluor 555, beta2-spectrin-Alexa Fluor 488, map2-DyLight405 acquired with NSPARC and Plan Apo Lambda D 100x oil.

Courtesy: Christophe Leterrier, NeuroCyto, INP, Marseille

Sensitive from Visible to Near-Infrared



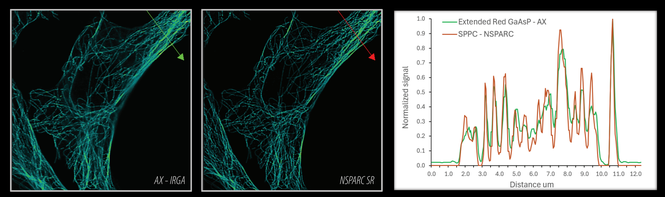
Fly wing with GFP-labeled neurons. Pseudo color image z-color coded. Zoom in image shows micro hairs. Nerves are better visible with 100x imaging.

Courtesy: Nikky Corthout (imaging) and Hélène Roberge (sample), VIB BioImaging Core Leuven, BE

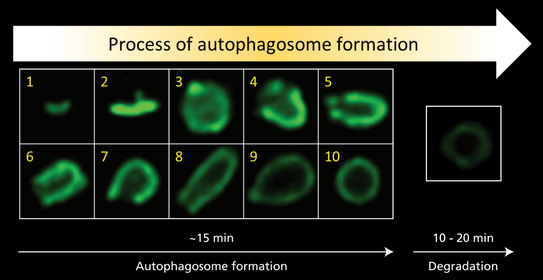
The fly wing is a transparent, thin, and inherently challenging sample to image. In this example, GFP-labeled neurons project throughout the wing. Imaging is complicated by the auto-fluorescent, uneven cuticle and the presence of multiple layers, including veins containing hemolymph and other cells. On the outer surface, micro hairs are visible as dark structures (see zoomed-in image).

A full overview of this massive sample is displayed on the left and represents a few square millimeters. Even at 25x magnification, axons are distinguishable, while neuronal projections are more clearly resolved at 100x magnification. Thanks to NSPARC's gentle imaging combined with superior silicon objective lenses, the entire sample was acquired without photobleaching, achieving optimal resolution and a high dynamic range.

NSPARC is a highly sensitive detector, even in the near-infrared (NIR) region. In this example, using Cell 4C NIR (Gattaquant), alpha-tubulin was labeled with Alexa Fluor 750, excited at 730 nm, and detected using either NSPARC or the extended-red GaAsP PMT of the AX detector. With high sensitivity, low noise, and enhanced resolution, NSPARC delivers superior image quality - offering improved resolution with signal-to-noise ratios comparable to conventional confocal imaging.



Live Imaging

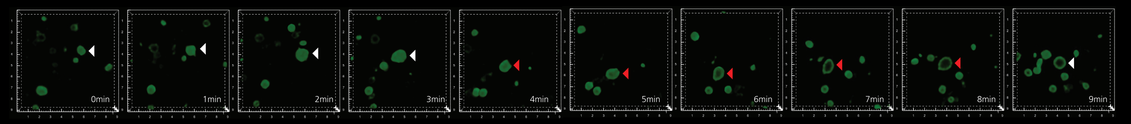


Autophagy is crucial for cellular balance, and its dysfunction is linked to cancer and neurodegenerative diseases. Despite extensive studies the dynamics of autophagosome formation remain unclear.

Since autophagosome formation is dynamic and requires nm resolution, NSPARC is optimal to accurately follow each steps of the autophagosome formation and precisely determine the membrane closure timing.

Tracking autophagosome formation and membrane closure was achieved with rapid imaging (1.5s/stack) avoiding photobleaching or cell damage, revealing significant 3D movement of the isolation membrane during autophagosome formation.

APP NOTE
Read the full application note here



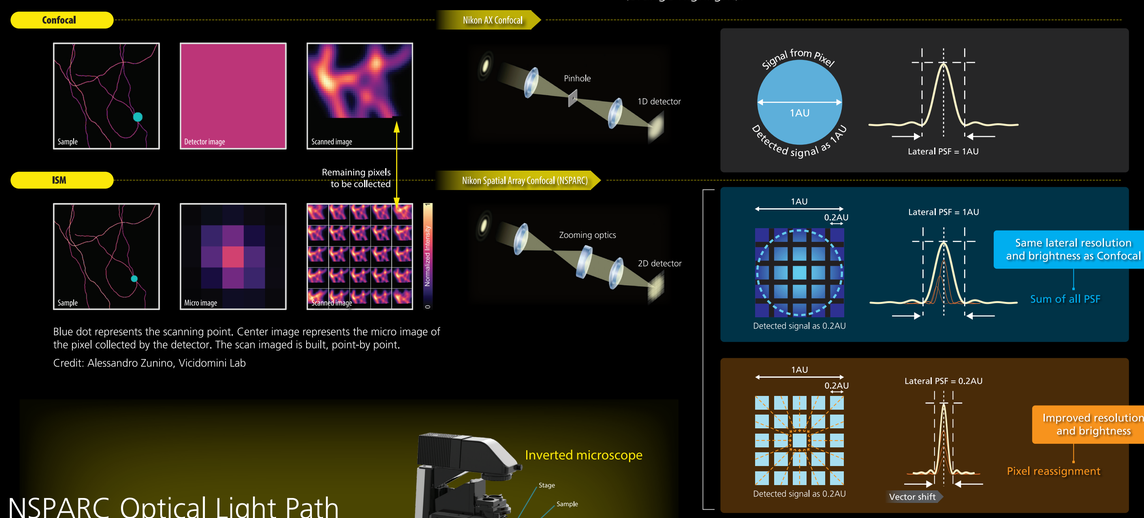
Mouse embryonic fibroblasts expressing GFP-LC3 were captured over 9min with Z-step: 0.15 µm, Z acquisition range: 2 µm with NSPARC and CFI Plan Apochromat Lambda D 100X Oil. Images were deconvolved using the Richardson-Lucy method. The arrow indicates a single 3D autophagosome. Dynamic changes in the closure of the isolation membrane were captured between 4 and 8 minutes (red arrow). Tick marks indicate position in µm.

Courtesy: Dr. Nobuo Noda and Dr. Yuta Ogasawara, Institute for Genetic Medicine, Hokkaido University

Image Scanning Microscopy (ISM) - The Principle

In confocal microscopy, a single detector (PMT) records one intensity value per position, forming the image point-by-point. In contrast, image scanning microscope (ISM) captures a 2D micro-image at each scan point with each detector element (SPPC) producing a slightly shifted point spread function (PSF).

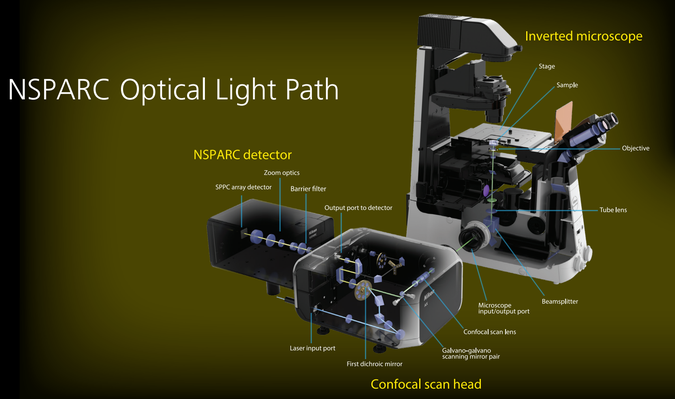
By summing each PSF from each element of the array, the lateral PSF will be equivalent to regular confocal PSF (blue highlight). However, by reassigning these shifts (vector shift), ISM increases image resolution (to 0.2 AU) without reducing signal brightness compared to conventional confocal imaging (orange highlight).



Blue dot represents the scanning point. Center image represents the micro image of the pixel collected by the detector. The scan imaged is built, point-by point.

Credit: Alessandro Zunino, Vicidomini Lab

NSPARC Optical Light Path



PRODUCT INFORMATION
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Shedding New Light On MICROSCOPY