APPLICATION NOTE

AX/AX R with NSPARC Confocal-based Super Resolution Microscope

3D Super-Resolution Imaging of Glial Cells in Cleared Mouse Brain Tissue using NSPARC

Glial cells play crucial physiological roles in the brain, including supporting neurons, and are implicated in neurodegeneration and lesion formation. For example, glial activation is observed in conjunction with the aggregation of misfolded proteins and neuronal cell death in neurodegenerative diseases like Alzheimer's disease. Investigating the relationship between the progression of neurodegenerative diseases and changes in cellular microstructures requires high spatial resolution imaging of brain tissue.

This application note presents a study in which fluorescently labeled, cleared mouse brain sections were imaged in 3D with four-color super-resolution confocal laser microscopy using the AX/AX R with NSPARC. We successfully visualized the 3D morphology of synapses and the fine processes of two types of glial cells in brain tissue simultaneously at high spatial-resolution.

Keywords: NSPARC, super-resolution, glial cells, astrocytes, microglia, synapses, tissue clearing, brain tissue

Glia and Synapse Imaging in Cleared Mouse Brain Tissue via NSPARC

A transgenic mouse brain, astrocyte-specific GFP expression, was perfused with PFA and then sectioned at 100 µm using a vibratome, followed by immunostaining against synaptic-marker and microglia-marker proteins with a nuclear dye. After the optical clearing process, the sections were covered with coverslips, and the cerebral cortex was imaged using a 100x silicone immersion objective on the NSPARC-equipped microscope. The combination of NSPARC and tissue clearing enabled detailed, simultaneous 3D visualization of synaptic markers and the morphology of astrocytic and microglial processes. In the entire field of view, thick astrocytic processes extending to grasp blood vessels and fine sponge-like processes were observed, along with intertwining microglial processes (Fig. 1A). At higher magnification, granular or ring-shaped synaptic markers surrounded by a meshwork of fine astrocytic processes were observed (Fig. 1B). Capturing this level of detail in 3D and multicolor is still challenging with conventional super-resolution microscopy or electron microscopy, highlighting the advantages of NSPARC.

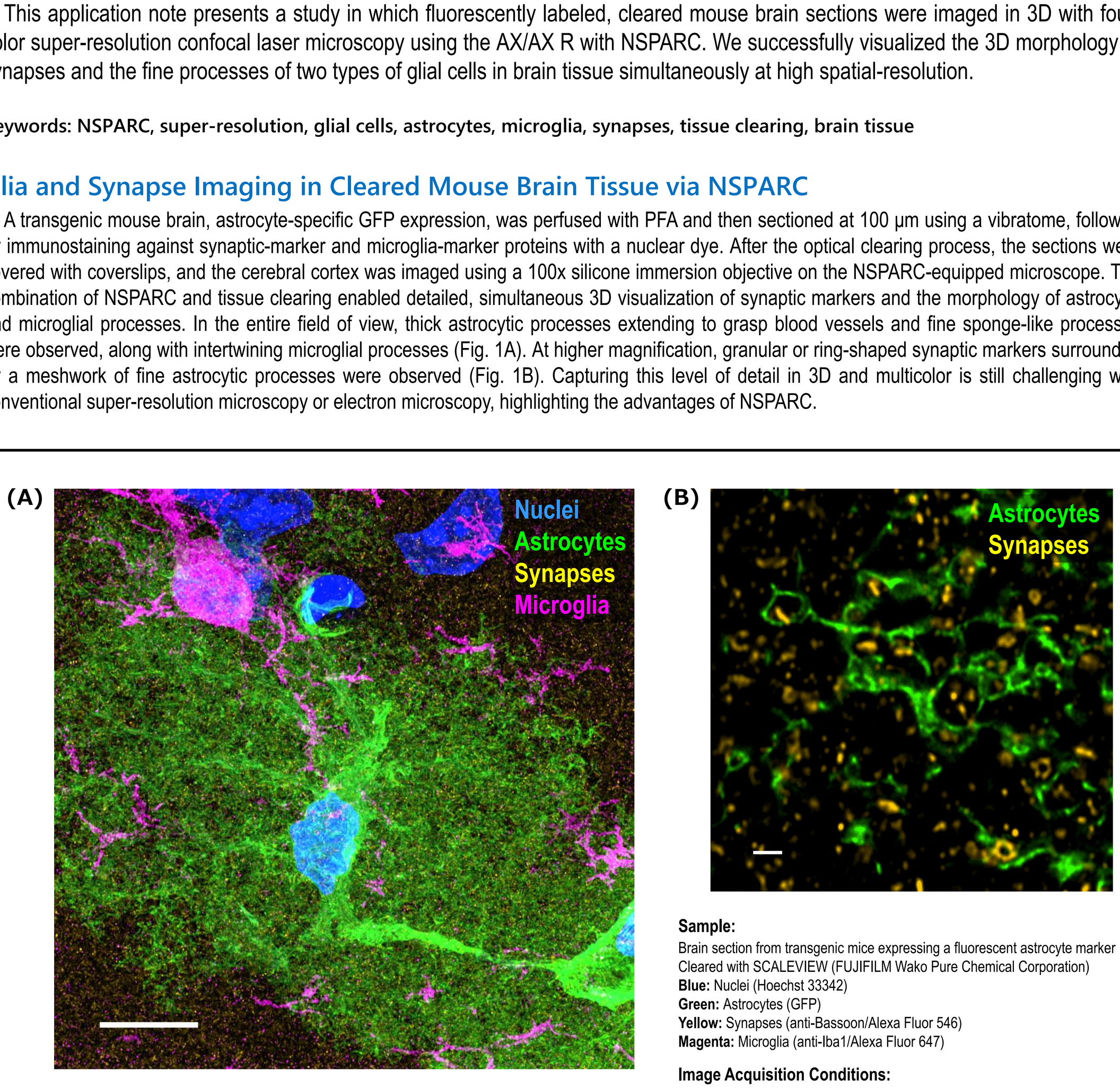

Figure 1. Two Types of Glial Cells and Synapses in Mouse Brain Tissue

(A) Maximum intensity projection (MIP) image of a cleared mouse brain section in the cerebral cortex (deconvolution applied). Scale bar: 10 µm
(B) Enlarged view of a single Z-slice. Scale bar: 1 µm

Sample:

Brain section from transgenic mice expressing a fluorescent astrocyte marker
Cleared with SCALEVIEW (FUJIFILM Wako Pure Chemical Corporation)
Blue: Nuclei (Hoechst 33342)
Green: Astrocytes (GFP)
Yellow: Synapses (anti-Bassoon/Alexa Fluor 546)
Magenta: Microglia (anti-Iba1/Alexa Fluor 647)

Image Acquisition Conditions:

Microscope System: Ti2-E + AX R with NSPARC
Objective: CFI SR HP Plan Apochromat Lambda S 100XC Sil, NA 1.35
Zoom: 3.00x
Scanner: Galvano
Excitation Lasers: 405/488/561/640 nm
XY: 4096 × 4096 pixels (59 µm × 59 µm, 0.014 µm/pixel)
Z: 35 slices (Range: 17 µm, Step: 0.50 µm)
Mode: SR

3D Visualization of Complex Structures using NIS-Elements Image Analysis

We used the NIS-Elements image analysis software, including the General Analysis 3 and NIS.ai modules, to segment microglia and astrocytes from the acquired images and visualize their 3D structures.

Microglial segmentation revealed the complex intermingling of microglial processes within the astrocytic domain (Figure 2A). To visualize the interaction of astrocytic processes with blood vessels and synapses, we selectively displayed synapses in close proximity to astrocytes, reducing visual clutter from the densely distributed clusters (Figure 2B).

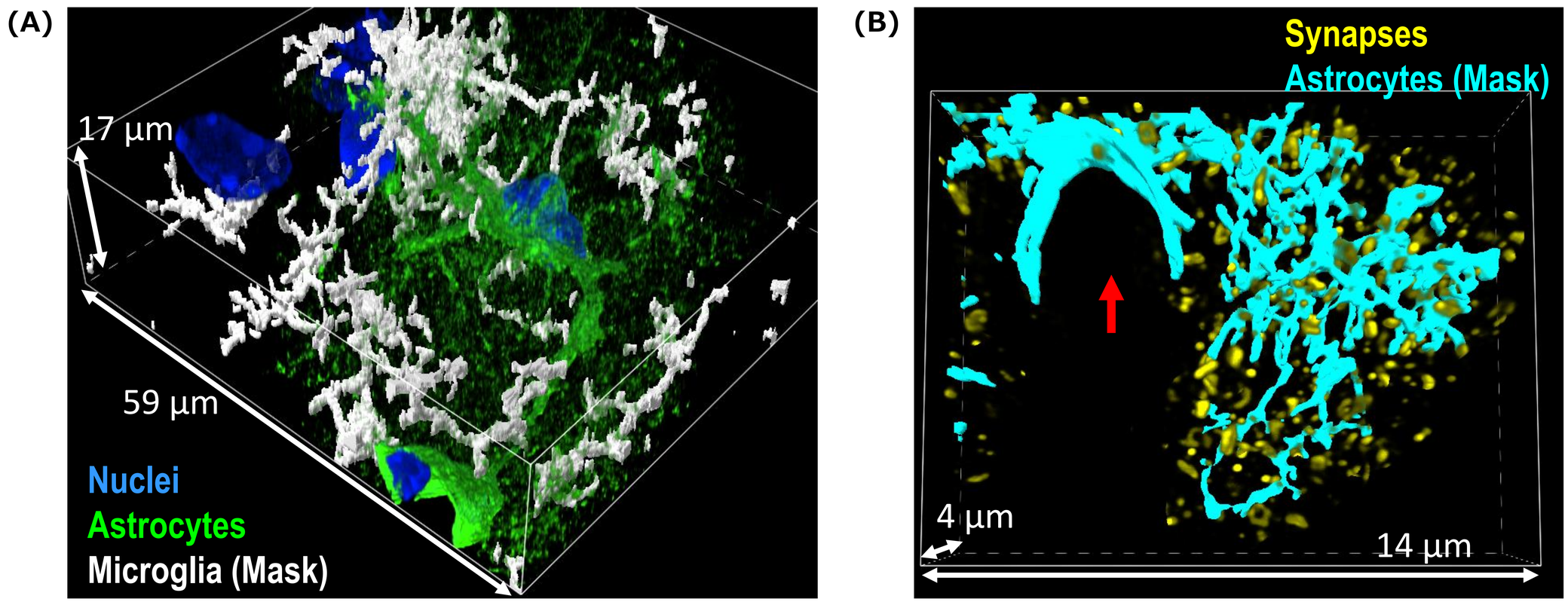

Figure 2. 3D Visualization of Microglia and Astrocytes using NIS-Elements

(A) 3D view of astrocytes (green) and segmented microglia (white).
(B) 3D view of synapses (yellow) and segmented astrocytes (cyan). Zooming in reveals astrocytic processes enveloping both putative blood vessels (red arrow) and synapses.

Video:

Advantages of Imaging Cleared Samples

Conventional light microscopy faces two key limitations for deep tissue imaging: the ~200 nm diffraction limit and light scattering/absorption, which degrades signal and limits penetration depth. Specialized techniques can overcome these, but often involve complex and time-consuming procedures.

The combination of tissue clearing and NSPARC allows for high-resolution 3D imaging with simplified sample preparation and standard confocal operation, enabling rapid data acquisition.

Table 1. Comparison of Methods for High-Resolution 3D Imaging of Tissues

	Electron Microscopy	Expansion Microscopy*	Clearing + NSPARC
Spatial Resolution	+++	++	++
Sample Preparation	+	+	+++
3D Imaging	+	+++	+++
Features	• Nanoscale resolution • Difficult to identify proteins and nucleotides with cellular structure	• Super-resolution with a standard light microscope • Signal loss problem due to physical expansion	• Commercial clearing kits is available • Easy multicolor 3D imaging

*Expansion microscopy (ExM) is a technique in which a sample is physically expanded using a swellable gel, enabling the visualization of fine structures.

Conclusion

The combination of tissue clearing and the AX/AX R with NSPARC facilitated simultaneous, high-resolution 3D imaging of synapses, astrocytes, and microglia in mouse brain tissue. This approach holds promise for advancing research on neurodegenerative diseases, such as Alzheimer's disease, by facilitating the study of protein localization with cellular microstructure.

Acknowledgements

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Footnote

Tissue Clearing

Tissue clearing is a histological method that reduces light scattering and absorption in biological tissues, rendering them transparent. This enables detailed, deep-tissue imaging with light microscopy. Clearing protocols typically involve lipid removal, depigmentation, and refractive index matching, often simplified by commercially available kits. Tissue clearing is applicable to a wide range of samples, including whole organisms, organoids, and tissue sections. Combined with 3D imaging, tissue clearing has become a powerful tool in neuroscience, cancer biology, developmental biology, and beyond.

Product information

AX/AX R with NSPARC Confocal-based Super Resolution Microscope

The super-resolution detector NSPARC, which has a 25-detector array, achieves even higher resolution with a high S/N ratio, without impairing the functions of the conventional AX/AX R confocal microscope.

Product information is here