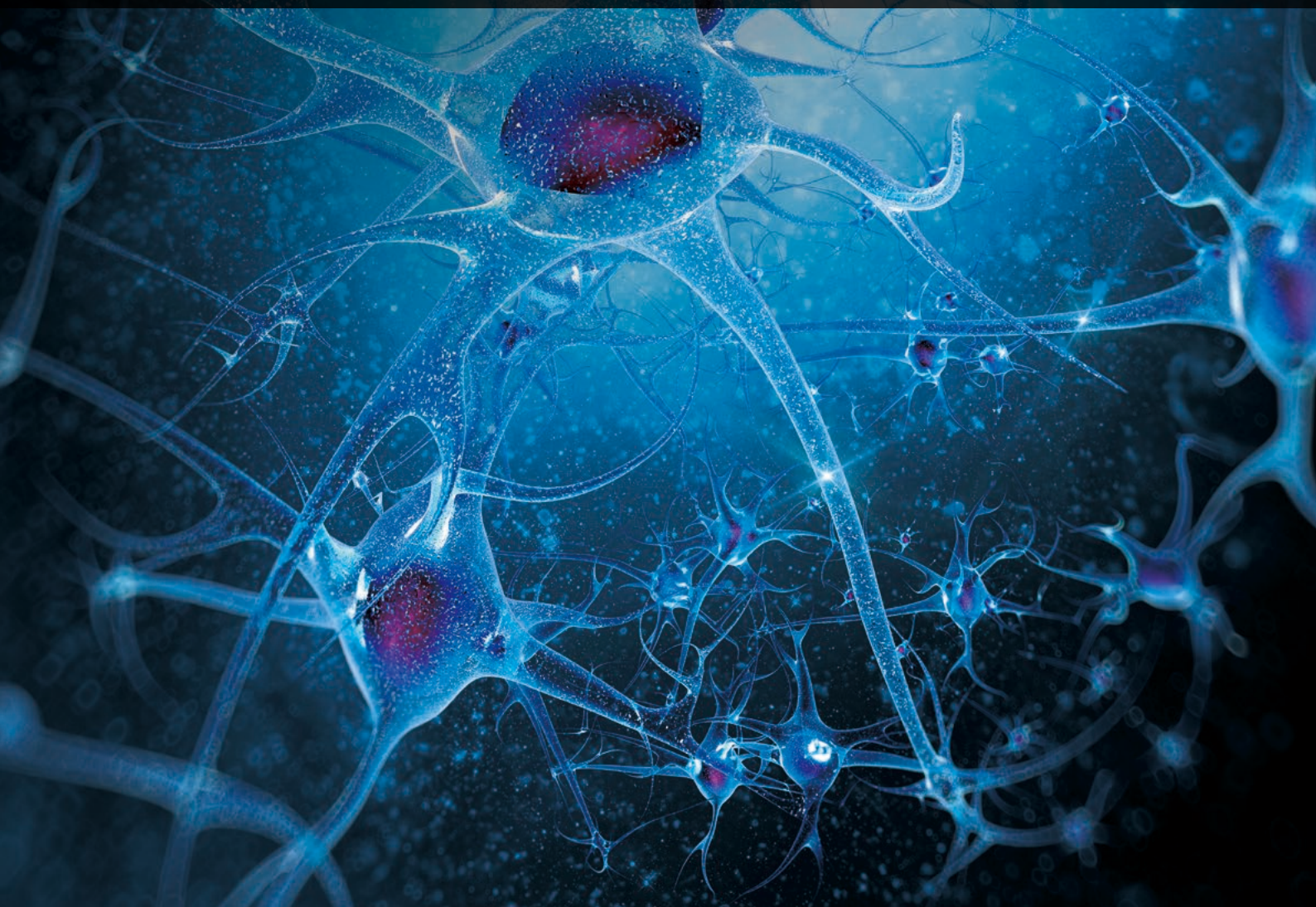
Nikon : Imaging without Limits

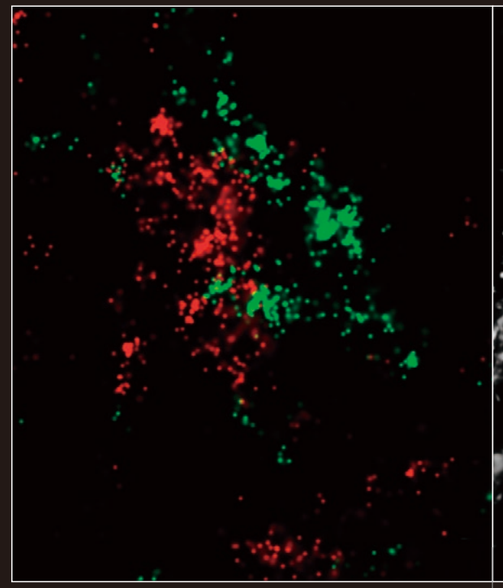
Neuroscience Edition



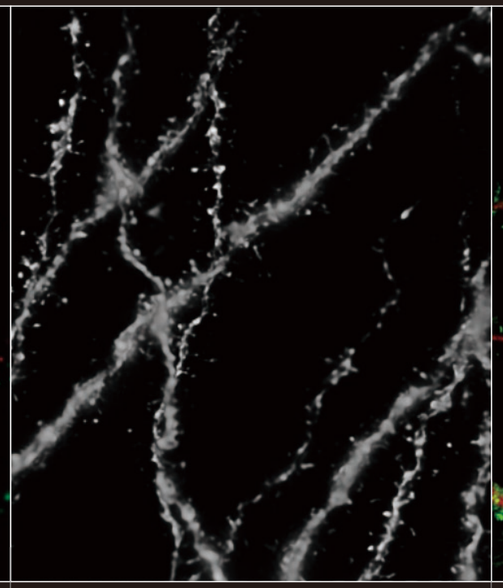
Explore neural networks from every angle, from the entire brain down to individual synapses

Nikon provides a variety of cutting-edge bio-imaging products that are driving outstanding developments in the field of neural research. With its highly renowned technologies and extensive experience in advanced imaging systems, Nikon supports morphological observations and functional analyses of neural networks utilizing various observation depths, scales, resolution and speeds.

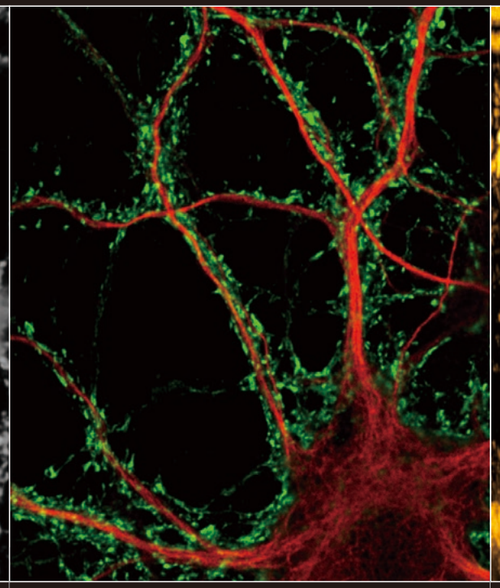
Pre- and post-synapses



Dendritic spines

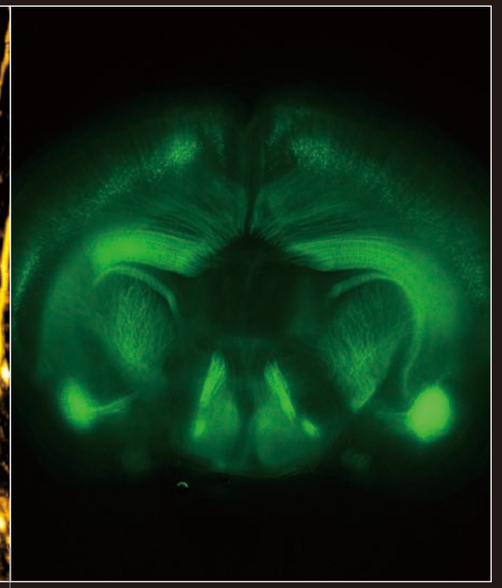


Dendrites



Cerebral cortex

Entire brain



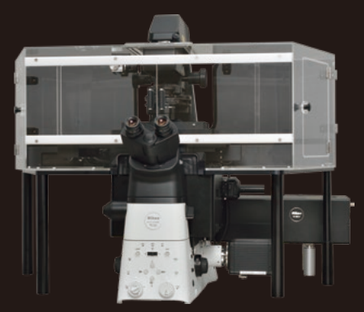
Super Resolution Microscope

Confocal Microscope

Two-photon Microscope

Stereo Microscope

N-STORM Super Resolution Microscope



N-SIM S Super Resolution Microscope

A1R HD25 Confocal Microscope

A1R MP+ Multiphoton Confocal Microscope

SMZ25 Research Stereo Microscope

Analysis of synapse dynamics by structured illumination microscopy

Shigeo Okabe, MD, PhD
Professor & Chair, Department of Cellular Neurobiology,
Graduate School of Medicine, The University of Tokyo

Nikon product:
N-SIM Super Resolution Microscope

Please summarize your research

In 1999, our group initiated live imaging of synapses using GFP-tagged postsynaptic molecules, such as PSD-95. Before the introduction of GFP-based synapse imaging, a widely accepted theory had been that synapses are stable and rigid cellular components with few structural alterations once they are formed. However, GFP-based synapse imaging provided evidence that synapses receive extensive remodeling and functional changes. After the introduction of two-photon imaging, research on structural remodeling of synapses further developed to direct observation of synaptic behavior in vivo. Our laboratory currently performs live imaging of synapses both in vitro and in vivo, together with development of new methods of quantitative analysis of synapse structure. We hope this approach leads to the construction of an integrated model that explains the stability and flexibility of synapses.

Why do you use an N-SIM system?

Among the available super-resolution approaches, SIM is advantageous in volume imaging of complex structures. STED and PALM/STORM are superior in resolution, but the limited speed of image acquisition and more extensive photobleaching intrinsic to these methods preclude their application in capturing dynamic morphological changes of dendritic spines, which receive most of the excitatory synapses in the forebrain pyramidal neurons. SIM is also flexible in multi-color imaging and allows us to capture images of both spine structures and clustering of synaptic molecules, which are essential information in understanding the molecular mechanisms of synapse formation.

Your research combines N-SIM with computational analysis of spine morphology. What is the merit of combining N-SIM image with computational analysis?

We found that the resolution of N-SIM is sufficient to record the structural details of synaptic structures. The high quality data obtained by N-SIM is ideal for further computational analysis of spine surface geometry and its regulation by synaptic activity. We expect this research direction will help us with the classification of spine synapses in an objective manner and the development of a new automated screening platform of chemical compounds that cure synaptic pathology in neurological and psychiatric diseases.

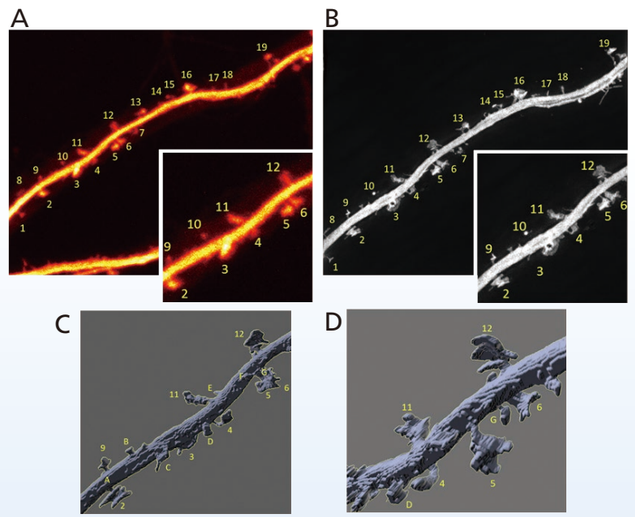
What are the outstanding aspects of Nikon and Nikon products?

Nikon is a leading company in imaging science with a long-standing partnership with biologists in Japan. We appreciate the high standard and stability of Nikon imaging products and have been fully satisfied with the service provided by Nikon N-SIM team, with its high quality and promptness. Our development of new analytical methods of spine structure could not have been achieved without support of Nikon.

What will be the future of microscopy, especially in the context of your work?

We are currently conducting imaging of synaptic structure both in vitro and in vivo. An amazing property of synapses is their stability and flexibility. Without synapse stability, we can not retain our memory. Without synapse flexibility, we cannot acquire new skills and learn from new experiences. A full understanding of these two contradictory properties of synapses requires quantitative data in nanoscale structures of synapses in vivo. I expect future technical development will enable in vivo nano-scale imaging of synapses.

A conventional confocal image (A) and a SIM image (B) of a dendrite and dendritic spines filled with GFP. Lower images (C and D) show surface data of the same dendritic segment. Numbers (1-19) indicate spines identified from the confocal image and letters (A-G) indicate additional spines only visible in the SIM image.



Cell-specific STORM Super-resolution Imaging Reveals Nanoscale Organization of Molecules

Istvan Katona, PhD
Laboratory of Molecular Neurobiology, Institute of Experimental Medicine of the Hungarian Academy of Sciences

Nikon product:
N-STORM Super Resolution Microscope

Please summarize your research

The brain is an extremely complex structure and in brain disorders like epilepsy, schizophrenia or autism, important cell-type-specific molecular changes occur. We and other research groups have discovered that in epilepsy, certain signaling pathways are upregulated in one cell type, whereas they are downregulated in another cell type. On a molecular level not only does the number of receptors or enzymes change, but their position also changes. We study these changes of the nanoscale spatial organization of molecules to understand how nerve cells talk to each other and how they are associated with particular neurological diseases.

Why do you use an N-STORM system?

Synapses are tiny structures; the distance between the presynaptic and the postsynaptic neuron is between 10 and 20 nm and the whole synapse is around 200 nm in diameter. STORM imaging is the most important tool for our research as it allows us to see the precise position of signaling molecules within the chemical synapse.
We choose STORM over other super-resolution approaches such as SIM or STED as it is the only technique that enables us to get the precise localization of individual molecules. Additionally, our lab has a background in electron microscopy and STORM is conceptually the closest to the conventional immunogold electron microscopy we have been doing for twenty years, so it was easy for us to implement in our research.

You combine N-STORM with confocal imaging; what are the benefits of combining these imaging modalities?

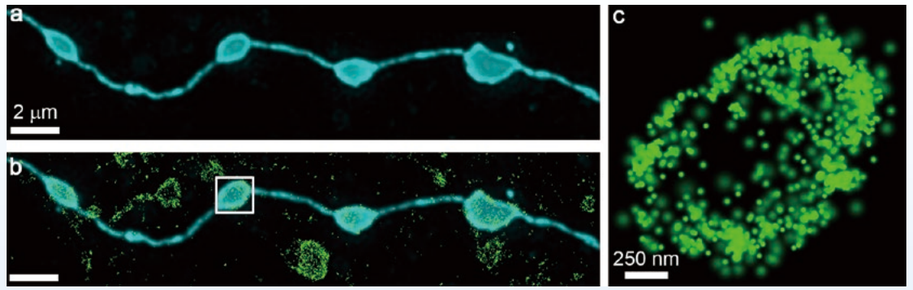
STORM imaging alone allows us to measure the position and distribution of important signaling molecules at the nanoscale level, but this data alone is like looking at stars in a dark sky. We need to use confocal microscopy to show in which context these molecular changes are taking place. In short, confocal imaging allows us to put molecular STORM data back into a subcellular and cellular context.
The combination of confocal microscopy and super resolution microscopy gives us the possibility to correlate physiological data, morphological data and molecular data. We record the physiological activity of neurons doing patch clamp experiments and we can study the activity of signaling pathways. During the recording, we also introduce a morphological dye into the cell via the patch clamp electrodes that enables us to identify the neuron, out of the several million in our brain circuit, from which we obtained the physiological data. After the physiological experiments we use confocal imaging for the morphological characterization of the cell type and the synapse. Then, we switch to STORM mode to gather information on the signaling molecules responsible for the physiological signaling. So, in this way we can correlate the molecular data, the anatomical data and the physiological data from the very same synapse. And this is very exciting!

What are the outstanding aspects of Nikon and Nikon products?

We had the very exciting opportunity to test the first N-STORM system in Europe, which allowed us to generate fantastic data. The N-STORM development team in Japan was always very helpful to respond to our questions on how to exploit this state-of-the-art technology in the most efficient manner. In addition, we really appreciate the very high level of service we get from Nikon Austria and the Hungarian service teams. STORM is an amazing technology, but these are very complex instruments so having a fast and high quality service is very important to ensure the smooth running of our research.

What will be the future of microscopy, especially in the context of your work?

We are very excited to get N-STORM 5.0 as we really look forward to performing live imaging in neuronal preparations to investigate how signaling molecules change their position in association with synaptic plasticity.
In my opinion, one very exciting innovation for us would be to use lateral illumination to excite only those fluorophores we do the imaging with; for example combining the light sheet approach with STORM imaging would certainly extend the usefulness of STORM technology into uncharted scientific territories.



Correlated confocal-STORM images demonstrating cannabinoid receptor localizations (green) on axon terminals of a hippocampal GABAergic interneuron (cyan). The images were taken on a combined N-STORM/C2 confocal system
Photos courtesy of: Dr. Barna Dudok, Laboratory of Molecular Neurobiology, Institute of Experimental Medicine of the Hungarian Academy of Sciences

Single and multi-photon imaging of neural networks in epilepsy

Steve Danzer, PhD
Director, Center for Pediatric Neuroscience
Associate Professor, Departments of Anesthesia and Pediatrics
Cincinnati Children's Hospital Medical Center
University of Cincinnati

Nikon product:
A1R MP Multiphoton Confocal Microscope

Please summarize your research

My laboratory is focused on elucidating the basic mechanisms that underlie the development of temporal lobe epilepsy. For the last decade, we have been testing the hypothesis that aberrant integration of hippocampal dentate granule cells mediates temporal lobe epileptogenesis. We have demonstrated that aberrant granule cells are present in the epileptic rodent brain and correlate with disease severity. We have demonstrated that genetically introducing aberrant granule cells can cause epilepsy in otherwise normal animals, and we have shown that ablating aberrant granule cells reduces disease severity in animals with established epilepsy. For all of these studies, we rely heavily on neuroanatomical approaches. Following the premise that "neuronal form predicts neuronal function," anatomical studies are a critical component of our research program. Findings are used to generate and test hypotheses, and guide genetic, physiological and functional studies.

Why/how do you use the A1R MP system?

Epilepsy is a disease of altered neuronal networks. To understand how these altered networks produce seizures, it is necessary to understand connectivity under both normal and pathological conditions. MRI techniques can reveal entire brains in living animals and patients and have produced great insights into how epilepsy changes the brain, but the technique lacks the resolution needed to elucidate the underlying circuitry. Traditional microscopy techniques can reveal circuits with high resolution, but are typically focused on relatively discrete samples of brain regions in thin tissue slices. The A1R MP system fills the gap between these approaches, making it possible to examine entire brain structures (or entire brains in smaller animals) in living animals at a level of resolution sufficient to reveal neuronal circuitry: cell bodies, axons, dendrites and dendritic spines. This tool is revolutionizing our approach to epilepsy research by allowing us to begin to examine entire circuits rather than small neuronal populations.

What are the outstanding aspects of Nikon and Nikon products?

We have been impressed with the ease of use, reliability and quality of Nikon products. The systems are capable of collecting large data sets extremely quickly, with high sensitivity, and excellent signal-to-noise. The software platforms are straightforward to understand and offer diverse acquisition methods. Students and fellows entering the lab are able to become proficient with minimal training. Technical support has been outstanding.

What will be the future of microscopy, especially in the context of your work?

Microscope systems are now capable of generating huge amounts of high resolution data of large brain structures. Using these systems, we have generated single-cell (and higher) resolution images of entire, intact hippocampi. Still lacking, however, are efficient and accurate approaches to analyze these large data sets. Automated software programs have made great progress in analyzing and extracting key measures from some types of images, but tedious and time-consuming work by students and postdocs still remains the gold standard. It is my expectation the automated programs will eventually be able to quantify the neural networks revealed in these images, at which point the field of neuroanatomy will allow investigators to examine entire brains for neuroanatomical changes. Because of the time consuming nature of current analysis approaches, neuroanatomical studies tend to focus on restricted brain regions, cell types or subcellular compartments. This approach, while fruitful, still amounts to "looking under the streetlight." The microscope technology allowing us to look everywhere now exists. Once the analysis side of the equation catches up, I expect we will see an explosion of exciting discoveries throughout the brain.

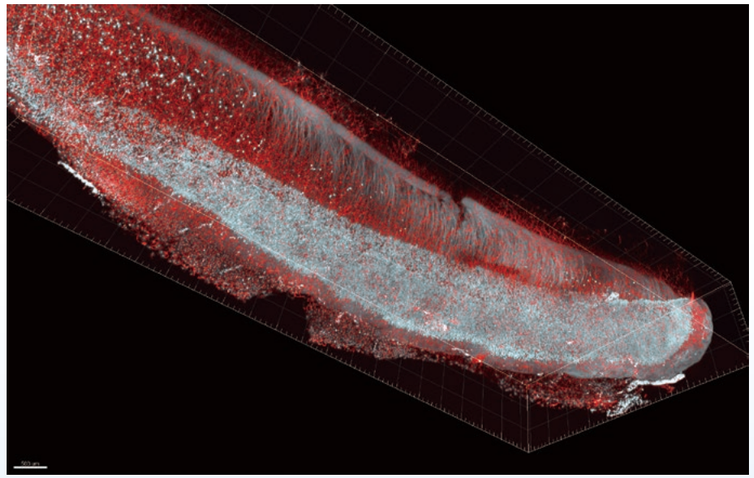
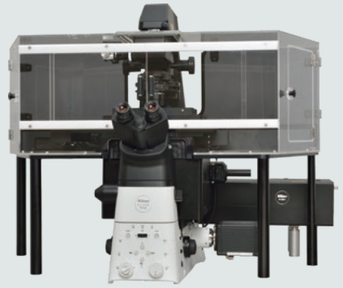


Figure Legend: Distribution of blood vessels and abnormal dentate granule cells in an epileptic mouse. Shown is the hippocampus from one hemisphere of a Gli1 - CreERT2, PTENfl/fl, TdTomfl/wt mouse with selective loss of PTEN only in dentate granule cells (gray) born after post-natal day 14. Blood vessels were labeled by intra venous injection of a fluorescent tomato lectin (red) shortly before sacrifice. Image by Mary Dusing and Candi LaSarge.

Nikon Imaging Products for Neuroscience Research

N-SIM S Super Resolution Microscope



Used for: Dendrite spine morphology
Capturing living nerve cell dynamics
Synapse counting and morphology

Features: Super-resolution up to approx. 115nm
High-speed live super-resolution imaging at 15fps
Low phototoxicity
Compatible with tissue clearing imaging

N-STORM Super Resolution Microscope

Used for: Pre- and post-synapse separation
Imaging of nano-domain distribution in the spine
Capturing of accurate co-localization of molecules in synapse

Features: Ultra-high resolution up to 20nm in xy and 50nm in z
Multicolor super-resolution imaging at the molecular level

A1R HD25 Confocal Microscope

Used for: Live nerve cell imaging
High speed imaging of Ca^{2+} dynamics
High content screening using nerve cells
Stitching of brain slice images

Features: Fast resonant scanner up to 720fps
Photo-activation imaging modality
High resolution 1K resonant imaging with large 25 mm FOV

A1R MP+ Multiphoton Confocal Microscope

User for: Ultra-deep in vivo neuronal imaging
High speed imaging of Ca^{2+} dynamics
Capturing of nerve cell elongation
Neural network analysis

Features: 1300nm IR pulse laser compatible
Fast resonant scanner up to 720fps
Compatible with both multiphoton excitation and visible confocal microscopy
High resolution 1K resonant imaging

SMZ25 Research Stereo Microscope

Used for: Embryo brain observation
Protein expression analysis
Sliced whole brain imaging

Features: Large FOV of 70mm
World largest zoom range of 25:1
Dedicated high resolution objectives

NIS-Elements Imaging Software

NIS-Elements

Used for: Neuron tracing analysis
Spine morphological analysis
High content screening
Image resolution enhancement

Features: Integrated control of various devices for imaging
Optional software packages for High Content Analysis (HCA) and Enhanced Resolution (ER)

Objective Lenses

Used for: Super-deep imaging
Super-resolution imaging for synapse analysis
Imaging of clearing processed samples

Features: Extremely long working distance for two-photon confocal imaging
Highest available NA for super-resolution imaging
Compatible with various immersion mediums
Equipped with a correction collar for a wide range of refractive indices

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. November 2018 ©2018 NIKON CORPORATION

⚠ WARNING	TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.

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*Products: Hardware and its technical information (including software)

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