



Shedding New Light On **MICROSCOPY**



# Silicone Immersion Objective Series

Perfect for imaging 3D cell cultures and thick tissue samples. Silicone lenses enable visualization of cellular dynamics clearly and brightly when imaging at depth.

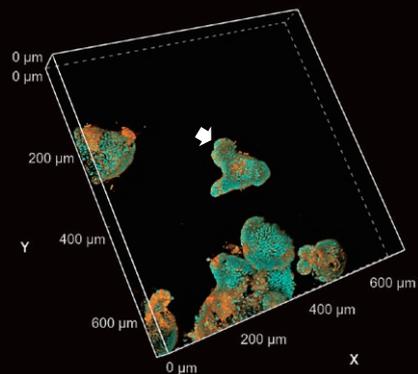
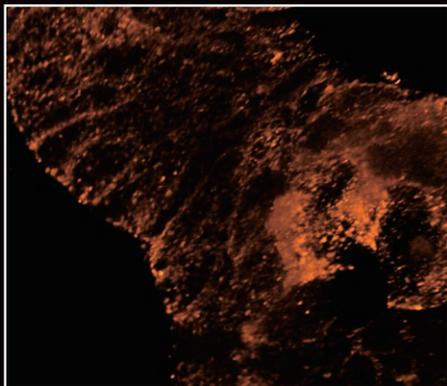
Research applications in neuroscience and cell biology using brain tissue, spheroids, organoids, and 3D cultures continue to push the limits of imaging in thick specimens. The need to image deep into such samples has never been more apparent. Nikon's silicone immersion objectives enable clear observation with high signal-to-noise deep into living tissue. The silicone immersion lens series features wide fields of view, high resolution, and evaporation-resistant oil, facilitating observations with ease.

With the addition of the new 60X objective, which employs newly developed glass for enhanced chromatic aberration correction, quantitative imaging in thick living specimens has never been more attainable.



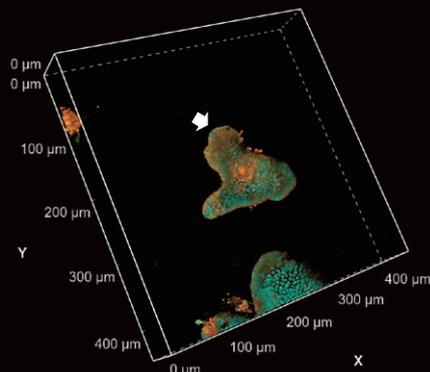
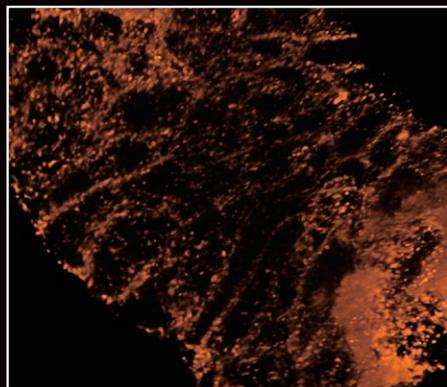
# Efficiently switch magnification for macro-to-micro imaging without changing the immersion medium

**25X** SIL25X/1.05  
Zoom 1X, Pixel 0.69 $\mu$ m



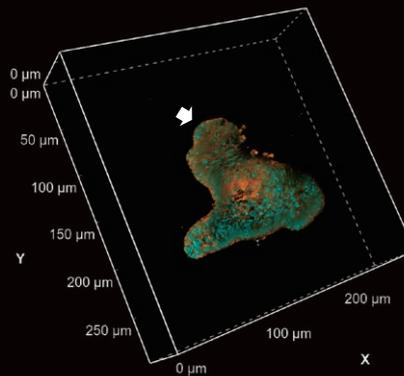
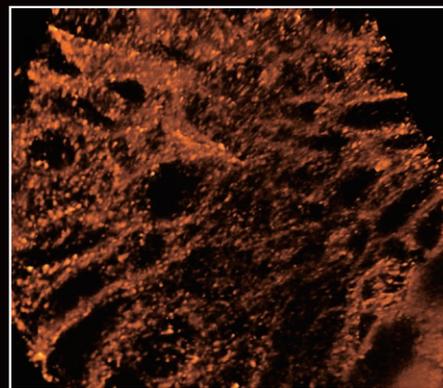
SIL25X/1.05  
Zoom 5X, Pixel 0.14 $\mu$ m

**40X** SIL40X/1.25  
Zoom 1X, Pixel 0.43 $\mu$ m



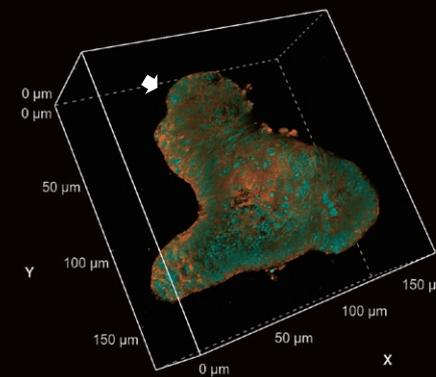
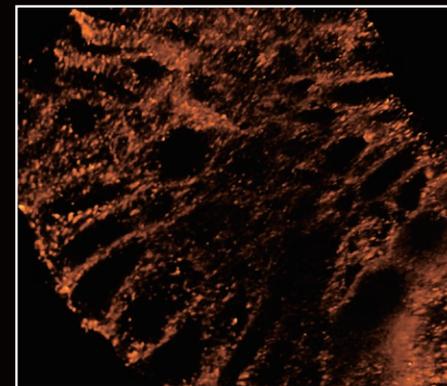
SIL40X/1.25  
Zoom 4X, Pixel 0.11 $\mu$ m

**60X** SIL60X/1.30  
Zoom 3X, Pixel 0.10 $\mu$ m



SIL60X/1.30  
Zoom 1X, Pixel 0.29 $\mu$ m

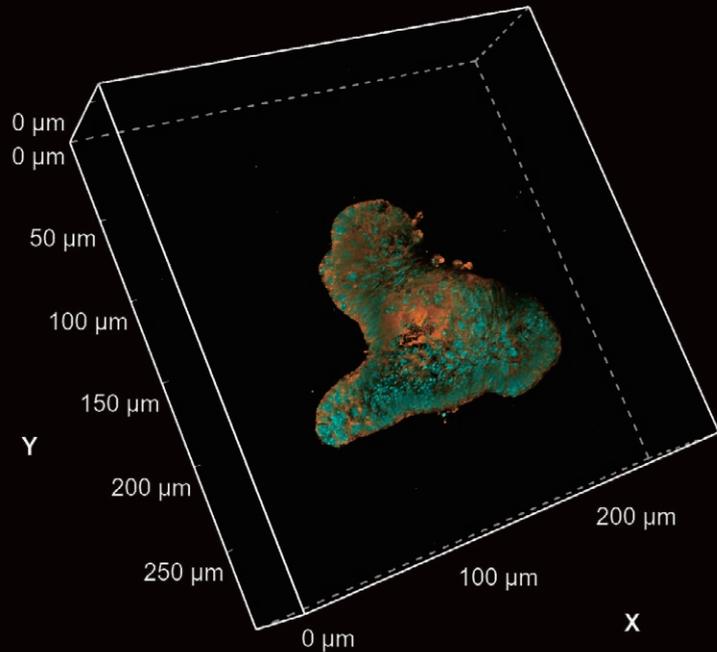
**100X** SIL100X/1.35  
Zoom 1.6X, Pixel 0.11 $\mu$ m



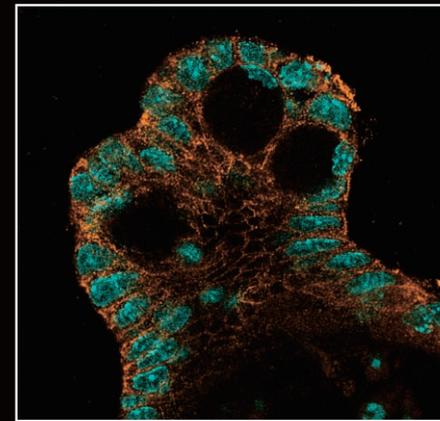
SIL100X/1.35  
Zoom 1X, Pixel 0.17 $\mu$ m

Small intestine enteroid (Cadherin: Alexa Fluor<sup>®</sup> 555, Nuclear: DAPI)  
Images courtesy of: Dr. Tokiyoshi Ayabe, Innate Immunity Laboratory, Department of Cell Biological Science, Faculty of Advanced Life Science, Graduate School of Life Science, Hokkaido University, Hokkaido University

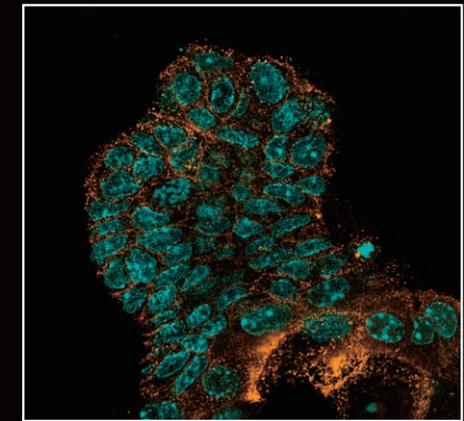
# Make high-resolution deep observations of thick specimens such as 3D cell cultures, organoids, and tissues



Small intestine enteroid (Cadherin: Alexa Fluor® 555, Nuclear: DAPI)  
Images courtesy of: Dr. Tokiyoshi Ayabe, Innate Immunity Laboratory, Department of Cell Biological Science, Faculty of Advanced Life Science, Graduate School of Life Science, Hokkaido University Science, Hokkaido University



2D image surface

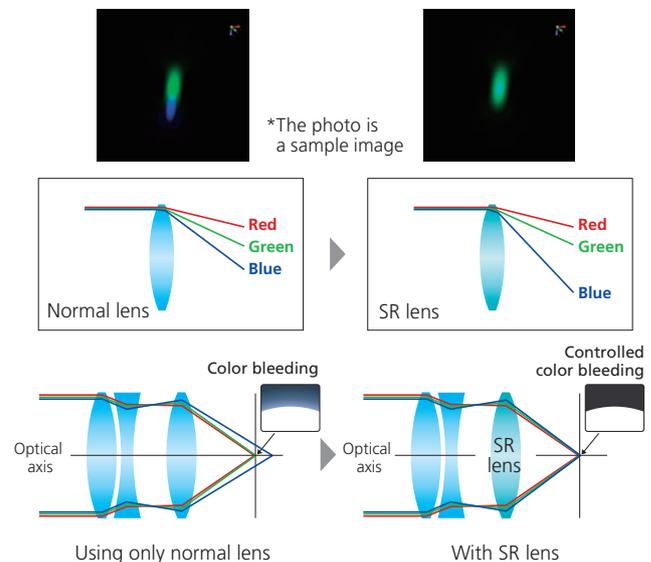


Deep 2D image

## Newly developed Short-wavelength Refractive (SR) glass

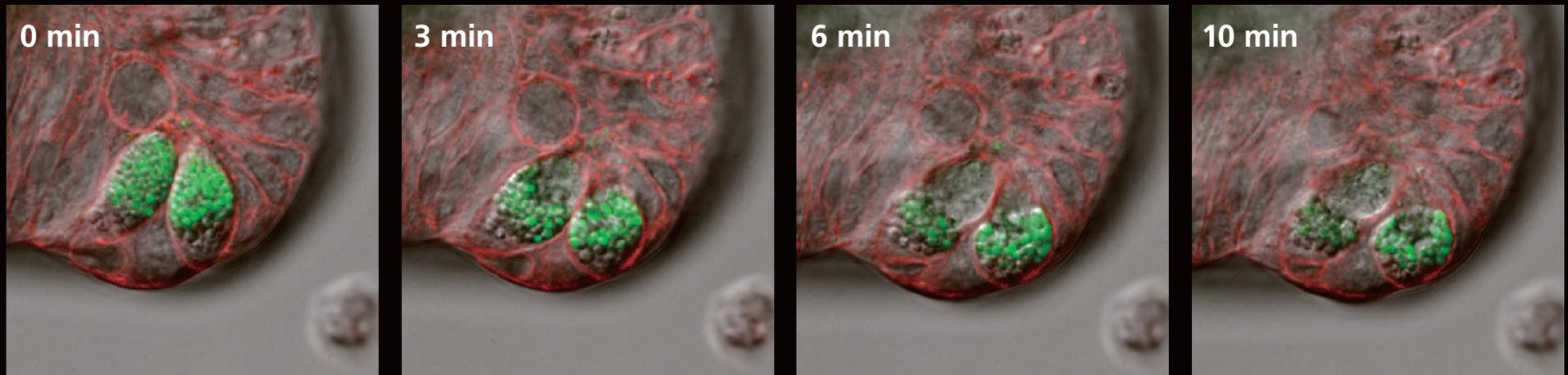
The new silicone immersion 60X objective employs high- and specialized-dispersion glass that was independently developed by Nikon and possesses extra low dispersion properties.

By refracting short-wavelength light to a higher degree, it is possible to collect a wider range of wavelengths, resulting in significantly enhanced chromatic aberration correction. In addition to axial chromatic aberrations, lateral chromatic aberrations can also be corrected.

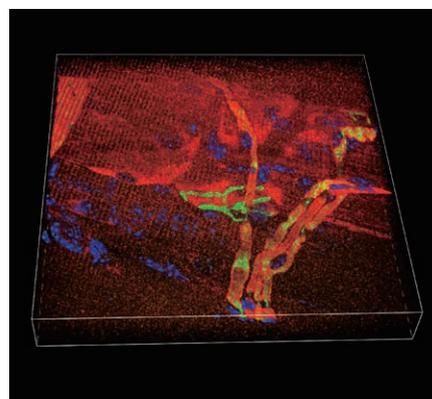


# Observe live samples over long periods of time

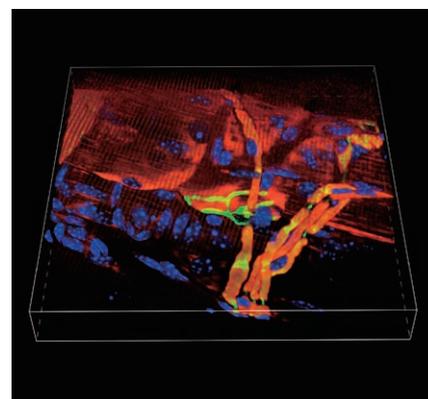
It is also best suited for long time-lapse imaging of live cells without the immersion medium evaporating because silicone oil has low volatility even at 37°C.



Time-lapse imaging of enteroid (25X) Images courtesy of: Dr. Yuki Yokoi, Dr. Kiminori Nakamura, and Dr. Tokiyoshi Ayabe, Innate Immunity Laboratory, Department of Cell Biological Science, Faculty of Advanced Life Science, Graduate School of Life Science, Hokkaido University



Before Denoise.ai processing



After Denoise.ai processing

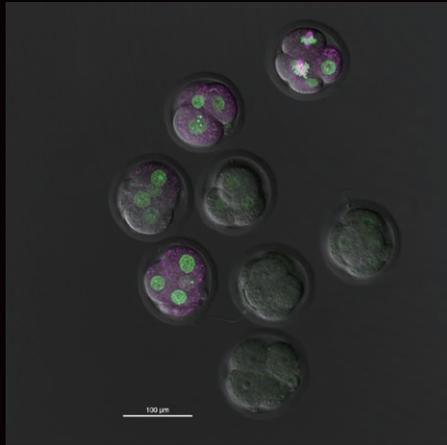
By using the Denoise.ai in conjunction with the AX confocal microscope, you can acquire even sharper images.

Details of examples are included in the application notes.

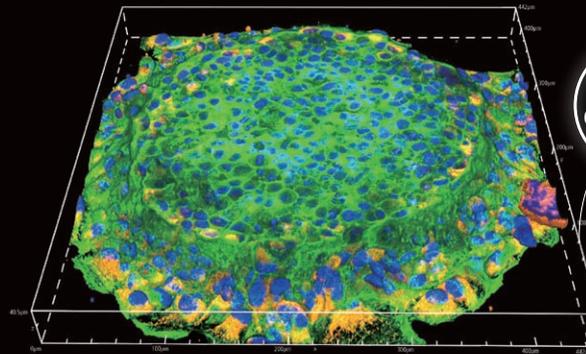
- High-speed, high-resolution 3D imaging using resonant scanner and Denoise.ai
- The Advantages of Resonant Scanning with Ultra Short Laser Exposure Times in Live Imaging
- In vivo Confocal Imaging of Mouse Organs that Clearly Captures Fast Dynamics (p-6)
- 3D Imaging of Intestinal Organoid (p-6)



# Useful for a variety of research applications



Fertilized mouse embryo  
Image courtesy of: Dr. Yoshiteru Kai,  
Reproductive Medicine Research Center,  
YAMASHITA SHONAN YUME CLINIC



Spheroid

Drug discovery

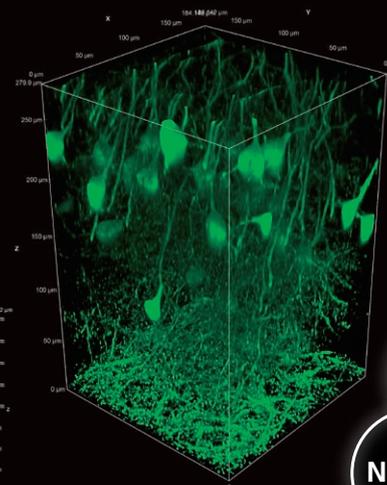


40X

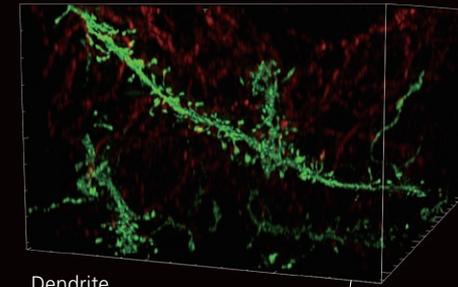
Regenerative medicine



Small intestine organoid  
Image courtesy of: Dr. Hidenori Akutsu and  
Dr. Tomoyuki Kawasaki of the  
Department of Reproductive Medicine,  
Center for Regenerative Medicine,  
National Center for Child Health and Development



Mouse nerves  
Images courtesy of: Lin Daniel,  
PhD. SunJin Lab Co.



Dendrite



100X

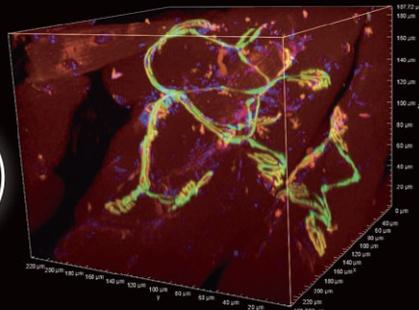
Neuron

Growth



25X

Neuron



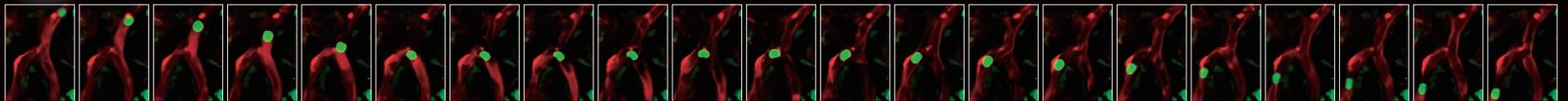
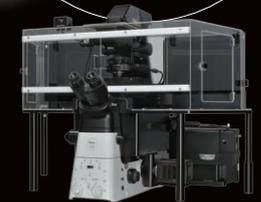
Mouse synapse

Drug discovery



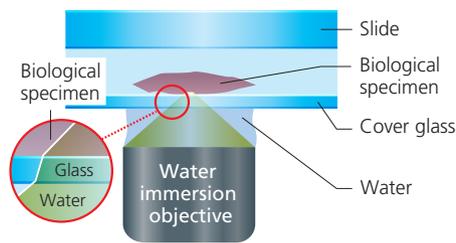
60X

Neuron



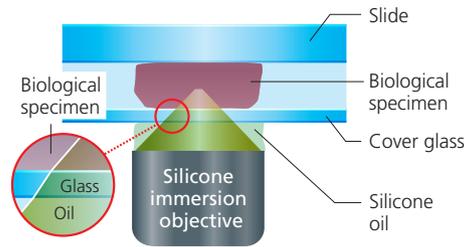
Neutrophil flowing in blood vessel (time-lapse) Images courtesy of: Professor Masaru Ishii, Department of Immunology and Cell Biology, Graduate School of Medicine, Osaka University

# Comparison of immersion objective types



## Water immersion objective features

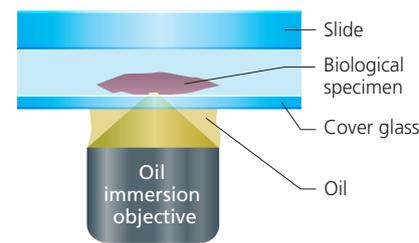
- Best for observing cultured cells and other specimens in culture solution because there is little spherical aberration as the refractive index is close to that of cultured cells and culture solution
- Best for in vivo observations and live tissue fragments because it is highly biocompatible as the refractive index is close to that of biological fluids
- Also effective with water immersion dispensers when it is necessary to supply immersion fluid, such as for observations of multiple points in multiple wells



## Silicone immersion objective features

- Little spherical aberration when making deep observations because the refractive index is close to that of many 3D cell cultures (spheroid, organoid, etc.) and organs with high refractive index (liver, etc.)
- Best for long time-lapse observations because oil is stable and resistant to drying even at 37°C.

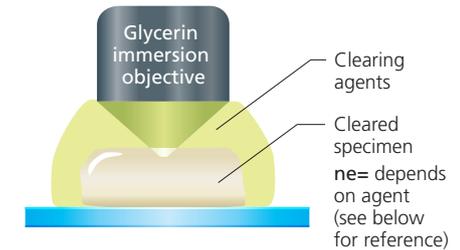
Oil used for silicone immersion objective  
Silicone immersion oil 30cc



## Oil immersion objective features

- Appropriate for observations that require high resolution because it has the highest NA of all immersion objective lenses
- Best for observations of sample surfaces (vicinity of cover glass) because its refractive index is close to that of cover glass ( $n_e=1.53$ )
- Best for observations of anchored specimens that use a mounting medium because the refractive index is close to that of many mounting mediums

For direct observations without cover glass.  
10X can also be used with cover glass



## Glycerin immersion (cleared specimen) objective lens features

- Possible to make deep observations of cleared specimens because glycerin has a refractive index close to many common clearing agents
- Can use with a wide range of clearing agents
- Can optimize spherical aberration correction to match the refractive index of the clearing agent using the correction collar

Examples of clearing agents appropriate for glycerin immersion (for clearing) objective lens  
Sca/e( $n=1.38$ )\*, CUBIC2( $n=1.48$ ), LUCID( $n=1.47$ ), FocusClear( $n=1.45$ ), CRARITY( $n=1.45$ ), SeeDB( $n=1.48$ )

## Comparison of immersion objective features

Immersion medium (possible magnification range)	Water (20X–60X)	Silicone (25X–100X)	Oil (40X–100X)	Glycerin (10X–20X)
Immersion liquid refractive index	Water ( $n_e=1.33$ )	Silicone ( $n_e=1.41$ )	Oil ( $n_e=1.52$ )	Clearing agent ( $n_d=1.33$ – $15.1$ ; 10X, $n_d=1.44$ – $1.50$ ; 20X) Glycerin ( $n_e=1.45$ )
Appropriate live samples for observation	Cultured cells Animal tissue and organs (mouse brain, etc.) with low refractive index Observations that must be biocompatible (in vivo observations, etc.) Observations that require multi-point observations of multiple wells (use of water dispensers)	3D cell cultures (spheroid, organoid, etc.) Animal tissue and organs with high refractive index (liver, etc.) Long time-lapse observations	Cultured cells and tissue fragments that require high resolution (sample surface)	—
Appropriate anchored samples for observation	Anchored cultured cells Anchored organ fragments with low refractive index (mouse brain, etc.)	Anchored fragments, such as organs with high refractive index (liver, etc.)	Cultured cells (with mounting medium) Tissue fragment (with mounting medium)	Cleared samples (spheroid, organoid, organs, etc.)

\* Refractive index depends on the organism

## Silicone immersion objective specifications

Model	Immersion	NA	W.D. (mm)	Cover glass thickness	Correction ring	Bright field	Dark field	DIC	Phase contrast	Polarizing	Fluorescence		Ti2-E PFS
											UV	Visible light	
CFI Plan Apochromat Lambda S 25XC Sil	Sil	1.05	0.55	0.11–0.23	✓	✓	Oil	✓		✓	✓	✓	
CFI Plan Apochromat Lambda S 40XC Sil	Sil	1.25	0.30	0.13–0.21 (23°C) 0.15–0.23 (30°C)	✓	✓	Oil	✓		✓	✓	✓	
CFI Plan Apochromat Lambda S 60XC Sil	Sil	1.30	0.30	0.15–0.19	✓	✓		✓		✓	✓	✓	
CFI SR HP Plan Apochromat Lambda S 100X Sil	Sil	1.35	0.31–0.29 (23°C) 0.30–0.28 (37°C)	0.15–0.19	✓	✓		✓		✓	✓	✓	

## List of application notes used

- High-speed, high-resolution 3D imaging using resonant scanner and Denoise.ai
- The Advantages of Resonant Scanning with Ultra Short Laser Exposure Times in Live Imaging
- In vivo Confocal Imaging of Mouse Organs that Clearly Captures Fast Dynamics
- 3D Imaging of Intestinal Organoid



List of application notes



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ISO 14001 Certified  
for NIKON CORPORATION

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

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### WARNING

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