Microscope Objectives for Bioscience
Tireless pursuit of the highest quality

Each Nikon microscope objective is precision-crafted to provide the highest level of clarity and overall optical performance. World-class Nikon objectives, including renowned CFI60 infinity optics, deliver brilliant images of breathtaking sharpness and clarity, from ultralow to the highest magnifications.

Exceptional performance born from advanced technology in glass formation and lens manufacture

Nikon’s extremely reliable high-tech products have incorporated the company’s cutting-edge optical and precision technologies since 1917. Over the past century, Nikon has researched and developed optical glass products in combination with optical designs for cameras, microscopes, IC steppers and others.

The front lens, which is the lens element at the tip of a high-power objective, is extremely small and has a distinctive shape. The lens is made of glass that meets Nikon’s strict material standards and designed with outstanding calculations.

A highly skilled expert must grind the lens by hand to meet the required high-precision standards and desired shape. The ground lens is then stringently and repeatedly checked using high-precision processing technology to ensure it meets Nikon’s compulsory high performance.
Development of CFI60 optical system

In 1996, Nikon developed the CFI60 (Chromatic aberration Free Infinity) optical system to meet demand for superior optical performance and system flexibility of biological microscopes for sophisticated and diverse research.

By using a tube lens focal length of 200mm and objectives having a parfocal distance of 60mm with a larger diameter and a 25mm thread size, Nikon succeeded in realizing both higher NA and longer working distances than ever before.

For these revolutionary optics, both axial and lateral chromatic aberrations have been corrected independently in the objective and tube lens without the aid of other components. The 200mm tube lens minimizes shifts between light rays as they pass through the fluorescence filter cube and DIC prism, creating a smaller angle between light rays passing through the center and those off axis to dramatically improve contrast.

A wide range of objectives to ensure reliable research results

Nikon provides the ultimate optical quality microscope objectives through highly-advanced technologies for precision optics production. These objectives offer highly reliable, high-quality images with maximum resolution and superior contrast for a wide range of applications, from routine tasks to cutting-edge bio science research.

Nikon Master Craftperson

Within the Nikon organization, there are dedicated personnel with the title of Nikon Master Craftperson. They have passed rigorous tests and possess a high degree of skill and expert knowledge, specifically for the production of objectives. Everyday, these “masters” utilize their techniques and knowledge to deliver unrivalled glass-based optical solutions.
The innovative coating technology enables bright, highly reliable image acquisition

In today’s bioscience research, it is becoming increasingly important to visualize minute cell structures and reveal mechanisms and the interaction of intracellular materials through fluorescent and confocal observations. To achieve more reliable imaging results, demand for bright objectives that can detect even the weakest fluorescent light has increased.

An objective is constructed with a number of lens elements to improve image quality and correct image distortion and aberration. However, due to surface reflection, light intensity weakens as light passes through each lens element. To reduce reflections and increase lens’ transmittance, lenses are coated.

**Nano Crystal Coat is Nikon’s superlative coating technology**

With its origins in Nikon’s semiconductor manufacturing technology, Nano Crystal Coat is an anti-reflective coating that assimilates ultra-fine crystallized particles of nanometer size. With particles arranged in a spongy construction with uniform spaces between them, this coarse structure enables lower refractive indices, facilitating the passage of light through the lens. These crystallized particles eliminate reflections inside the lens throughout the spectrum of visible light waves in ways that far exceed the limits of conventional anti-reflective coating systems.

Nano Crystal Coat eliminates ghost effects caused by red light, an achievement that has taken a long time, and effectively reduces flare effects caused by light entering the lens at an angle.
Nano Crystal Coat is Nikon’s superlative coating technology. These objectives provide chromatic aberration correction over a wide wavelength range from 405nm and are powerful enough for spectral imaging and simultaneous multi-wavelength acquisition. The LWD 20x WI λ S lens has an extremely wide chromatic aberration correction range of 405nm to 950nm and is suitable for multiphoton observation. The 40x WI λ S lens has an NA of 1.25, the world’s highest for a 40x water immersion objective. The 60x oil λ S lens offers high level chromatic aberration correction across the whole visible range and is a powerful tool for confocal spectral imaging and photostimulation.

Cutting-edge objectives with Nano Crystal Coat

These top-grade objectives employ Nikon’s exclusive Nano Crystal Coat technology and provide high transmittance up to the near-infrared range. Chromatic aberrations are highly corrected over a wide wavelength range, from ultraviolet to near infrared. The immersion objectives are the perfect choice for live-cell imaging, thanks to their incomparable high numerical aperture.

CFI Apochromat λ S Series

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CFI Plan Apochromat IR lens

With the world’s highest NA (1.27) for a 60x water immersion objective, this lens achieves a high level of resolution and sharp image acquisition. It corrects chromatic aberration up to 1,064 nm and accommodates laser tweezers.

CFI75 Apochromat MP lens

This lens provides a high numerical aperture of 1.10 while still maintaining a long working distance of 2.0mm. It corrects chromatic aberration up to the near-infrared range and has a ring that corrects chromatic aberrations depending on the depth of the specimen. Together with its 33° manipulator pipette access angle, it is ideal for deep imaging of live specimens using multi-photon excitation and physiology research applications.
Nano Crystal Coat guarantees optimum brightness

- Nano Crystal Coat enables remarkably high transmission up to the near-infrared region.
- Chromatic aberrations are corrected throughout a wavelength range from visible to near infrared. Bright, high-contrast images are captured during long-wavelength imaging, which is less phototoxic to live-cells.
- Unmatched chromatic aberration correction, resolution and image flatness ensure the capture of high-quality brightfield images.

Dramatically increased transmission rates over a wide wavelength range of up to near infrared.

Extended chromatic aberration correction from 435 nm to 850 nm enables the capture of clear images during multi-wavelength fluorescence imaging.

Image of HeLa cells labeled with four probes: Hoechst33342 (Nuclei, blue), Venus (Mitochondria, green), mCherry (α-tubulin, orange), Alexa 750 (Nucleoli, red)
Objective: CFI Plan Apochromat λ 100x oil
Photos courtesy of: Dr. Kenta Saito, the Center for Brain Integration Research, Tokyo Medical and Dental University
Dr. Kentarou Kobayashi, Research Institute for Electronic Science, Hokkaido University
Dr. Masahiro Nakano and Dr. Takeharu Nagai, the Institute of Scientific and Industrial Research, Osaka University

Near-IR dye image
Indocyanine green (ICG) fluorescence image of mouse auricularis blood vessels
Objective: CFI Plan Apochromat λ 20x
Excitation wavelength: 785nm
Peak emission wavelength: 832nm
Photo courtesy of:
Dr. Hirofumi Inoue;
Drs. Shigeki Higashiyama and Takeshi Imamura, Proteo-Science Center (PROS), Ehime University

Three-dimensional fluorescence image
3D fluorescence image of honey bee antenna
Objective: CFI Plan Apochromat λ 40x
DAPI: Cell nucleus
FITC: Dorsal branch of the antennal nerve
Rhodamine: Ventral branch of the antennal nerve
Specimen courtesy of: Dr. Hiroshi Nishino, Research Institute for Electronic Science, Hokkaido University
and Dr. Takeharu Nagai, the Institute of Scientific and Industrial Research, Osaka University
Reduced asymmetric aberration enables the capture of fluorescence bead images with even brightness during defocusing.

Nikon super-resolution technologies that go beyond the diffraction limit

The resolution of conventional optical microscopes is limited by diffraction to approximately 200 nm. Super Resolution Microscope N-SIM/N-STORM enables elucidation of the structures and functions of nanoscopic machinery.

N-SIM can achieve an image resolution of 115 nm and a temporal resolution of up to 0.6 sec/frame, enabling super-resolution, time-lapse imaging of live cells.

N-STORM can also achieve an incredible image resolution of approximately 20 nm, which is 10 times that of conventional optical microscopes.
Objectives with an unparalleled NA of 1.49

- Because of the unprecedented NA of 1.49—for use with a standard coverslip and immersion oil—these objectives enable the acquisition of bright, high S/N ratio images; so they are suitable for TIRF observation and live cell imaging.
- Both the 60x and 100x lenses utilize the spherical aberration correction ring to reduce deterioration in image quality caused by deviations in cover glass thickness or temperature fluctuations and provide optimal optical performance even at 37°C.
- High NA and the correction ring allow the acquisition of high-resolution, high S/N ratio images during TIRF observation, epi-fluorescence and confocal observation, as well as Nomarski DIC observation.
- The 100x objective can be optimally applied for laser tweezers microscopy.

TIRF for high-sensitivity fluorescent images with great signal-to-noise ratio

Nikon’s high NA TIRF objectives make it possible to introduce laser illumination at an incident angle greater than the critical angle (\( \theta \) \( c \)) for TIRF (Total Internal Reflection Fluorescence). In TIRF observation, light no longer propagates through the specimen, but sets up an evanescent field at the coverslip/specimen interface that can excite fluorescence in the specimen in an optical section less than 100nm. By exciting such a thin section within the specimen in contact with the coverslip, extremely high S/N data can be acquired.

Overview of Evanescent Wave Illumination

Much higher S/N ratio than a conventional model
Sample: Q-Dot
CFI Apochromat TIRF 100x oil, NA 1.49 (new product)
CFI Plan Apochromat TIRF 100x oil, NA 1.45 (conventional product)
**CFI Plan Apochromat VC Series**

**Essential for confocal observation such as DAPI**

- Top performance objectives with perfect correction of chromatic aberrations in the visible light range and excellent resolution throughout the view field.
- Perfect choice for multi-stained, fluorescence specimens and for brightfield and DIC observation.
- In addition to the correction range of the conventional Plan Apochromat series (435–660nm), axial chromatic aberration has been corrected up to the violet range (405nm), making these objectives highly effective for confocal applications.
- Observation of images with excellent brightness throughout the view field by minimizing the light loss around the edges and increasing resolution—a critical criterion for digital-image capturing.
- The 60x water-immersion type features high spectral transmittance, even in the 360nm wavelength ultra-violet range, making it perfect for fluorescence observation of living organisms.

**Comparison of conventional objectives and VC objectives**

With the conventional objective, DAPI fluorescence (blue) image may shift in the Z-axis direction due to axial chromatic aberration. With VC objective lens, on the other hand, as axial chromatic aberration has been corrected up to the violet range, DAPI fluorescence (blue) image shift in Z-axis direction is corrected and it is clearly seen that nucleus stained with DAPI is properly in a cell.

Fluorescence image of actin (green: Alexa 488, excitation: 488nm), mitochondria (red: Mito Tracker Organe, excitation: 543nm) and nucleus (blue: DAPI, excitation: 408nm) of HeLa cell. Consecutive cross-sectional XY and XZ images acquired with a confocal laser microscope and CFI Plan Apochromat VC 100x oil objective.

Water-immersion type CFI Plan Apochromat VC 60x WI objective is perfect for confocal observation of deep tissue

Overlaid consecutive cross-sectional scan within 108µm thickness range of a brain slice with neuronal cells expressing GFP.

Photo courtesy of:
Tatsuya Umeda, Department of Neuroanatomy, Faculty of Medicine, Yokohama City University and Dr. Shigeo Okabe, Department of Cellular Neurobiology, Faculty of Medicine, Tokyo University
Water-dipping Objective Series

New design for enhanced operability

- Long WD and high NA at any magnification.
- Sharper tips and broad approach angles provide improved accessibility for manipulator control.
- Aberrations are corrected even in the infra-red range with the high-magnification objectives, making them suitable for multi-photon imaging using infra-red light.
- 100xW objective with a correction ring that corrects spherical aberration induced by imaging depth or temperature fluctuations. With excellent infra-red transmission, this lens assures best quality images of even a thick specimen.

Water-dipping objective with low magnification, high NA and long working distance CFI75 LWD 16xW

Single objective covers a wide range of magnifications

- The 16x objective, when combined with FN1 microscope and dedicated magnification module, provides 5.6x, 32x, and 64x magnifications. As this single objective allows observation from a low magnification wide field to a high magnification high resolution field, it is ideal for patch-clamp experiments.
- With excellent IR transmission, this lens is also suitable for IR-DIC observation.
- With its high NA, the 16x objective provides superb image quality in combination with confocal laser microscopes.

Contrast doubled by reduction in halo

- The employment of an apodization phase ring reduces halo, which lowers the quality of phase contrast images. This improves the contrast of images to twice that achieved by a conventional product. This lens enables high-resolution observation of the minute structure in an unstained, low-contrast intracellular structure.
- With its high NA, this lens is also suitable for fluorescence observation.
- This lens is suitable for observation of the unstained structure and organelle of cultured cells as well as time-lapse observation of mitochondrial transport, growth cone and stress fiber.

Comparison with a conventional phase contrast objective

Images: from The 29th Optics Symposium (2004, Tokyo) 43-46
Photos courtesy of: Dr. Kaoru Kato, the National Institute of Advanced Industrial Science and Technology (AIST)
Objectives for brightfield observation

CFI Plan Apochromat Series
These high NA series feature superior image flatness and resolving power at the theoretical limit of today’s optical technology and are designed to correct all optical aberrations throughout the visible spectrum, from violet to red over the entire 25 mm field of view. The $\lambda$ series has high transmission rates and chromatic aberration correction up to near-infrared range, while the VC series corrects chromatic aberrations from 405 nm.

CFI Plan Fluor Series
Featuring an extra-high transmission rate, especially in the ultraviolet wavelength, and flatness of field, this series is designed for fluorescence observation and imaging. These objectives can function as multi-purpose objectives for brightfield, fluorescence, polarizing, and DIC observations.

CFI S Fluor Series
This CFI S Fluor series ensures a high transmission rate of ultraviolet wavelengths down to 340 nm for fluorochromes like indo-1, fura-2 and fluo-3. Also, these objectives have improved S/N ratios for short wavelengths and have high NA, making the fluorescence images they produce significantly sharper and brighter.

CFI Plan Achromat Series
Featuring an extra-high transmission rate, especially in the ultraviolet wavelength, and flatness of field, this series is designed for fluorescence observation and imaging. These objectives can function as multi-purpose objectives for brightfield, fluorescence, polarizing, and DIC observations.

Objectives for advanced modulation contrast observation

CFI Achromat Series
Correction of chromatic aberration, spherical aberration and coma has been dramatically improved, with significantly better image flatness across the 22mm field of view.

Nikon Advanced Modulation Contrast
Nikon has developed dedicated objectives for advanced modulation contrast. Colorless and transparent samples can be observed in high relief with a plastic dish, which is not possible in DIC observation. The direction of contrast can be matched to S Plan Fluor ELWD NAMC objectives, thereby allowing optimal contrast selection for techniques like microinjection and ICSI.
Objectives for phase contrast observation

CFI Plan Fluor ELWD Series
Newly developed broadband multilayer coating realizes high transmittance from near-ultraviolet (Ca2+) to near-infrared wavelengths, with improved chromatic correction. The correction collar ring allows these objectives to be used with a diverse range of culture vessels and specimen thicknesses. High-quality images with no aberrations can be obtained under a broad range of illumination techniques.

Apodized Phase Contrast Series
Nikon specifically developed this series for phase contrast observations by using its proprietary Apodization process to improve the objective’s phase ring. Cell division activities taking place within a specimen—hitherto often obscured by unwanted halos—can now be observed more clearly.

CFI Plan Apochromat Series for Phase Contrast
The λ series’ transmission rates and correction of chromatic aberrations have been improved and extended up to the near-infrared range. High NA, exceptional brightness, comprehensive aberration correction and superior flatness of field of view make these lenses ideal for the most demanding research projects.

CFI Plan Achromat Series for Phase Contrast
Nikon’s CFI Plan Achromat series provides incredible image flatness over the entire 25mm field of view, with chromatic aberration corrected throughout the entire visible spectrum. With incredible image sharpness, these objectives can be used for laboratory work as well as exacting research.

CFI Plan Fluor Series for Phase Contrast
These objectives are multi-purpose; they can be used for brightfield, fluorescence, or phase contrast observations. They facilitate high-quality fluorescence observation and provide exceptionally detailed resolution of minute structures in phase contrast. The use of phase contrast to find the desired portion of the specimen before switching to fluorescence observation is an excellent way to minimize fluorescence photo bleaching.

Objectives for apodized phase contrast observation

CFI Plan Fluor Series for Phase Contrast
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Objectives for inverted microscope Ti

CFI S Plan Fluor ELWD Series
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Nikon offers a wide variety of CFI objectives. To assist the user they are clearly marked with information on the objective barrel such as: which DIC module or Phase Ring to use.

(1) Magnification and Color Code
A color coded ring on the barrel identifies the magnification of the objective:

<table>
<thead>
<tr>
<th>Mag.</th>
<th>1x</th>
<th>2x</th>
<th>4x</th>
<th>10x</th>
<th>20x</th>
<th>40x</th>
<th>50x</th>
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<td>Color code</td>
<td>Black</td>
<td>Gray</td>
<td>Red</td>
<td>Yellow</td>
<td>Green</td>
<td>Light Blue</td>
<td>Light Blue</td>
<td>Cobalt Blue</td>
<td>White</td>
</tr>
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</table>

(2) Numerical Aperture (NA)
NA is the most important factor in defining the performance characteristics of an objective.

\[ NA = n \sin \theta \]

- n: the refractive index of the media at d-line (587nm)
- \( \theta \): Angle of half the cone of incident light that can enter or exit the top lens of the objective

For dry objective \( n = 1.000 \) (air)
For oil immersion objective \( n = 1.515 \) (oil)
For water immersion objective \( n = 1.333 \) (water)

The higher the NA, the higher the resolving power.

When the resolving power is defined as the power to distinguish the two points,

\[ R = \frac{0.61 \lambda}{NA} \]

If \( \lambda = 0.55 \mu m \) (Green light) and NA = 1.4,
resolving power \( R = \frac{0.61 \times 0.55}{1.4} = 0.24 \mu m \)

The higher the NA the brighter image.

Brightness: \( B \propto \left( \frac{NA}{Total\ Magnification} \right)^2 \)

The higher the NA, the shallower the depth of focus (DOF).

\[ DOF = \frac{n \lambda}{2NA^2} \]

(3) Working Distance
Working distance (WD) defines the distance between the top lens of the objective and the surface of the cover glass.

Cfis60 objectives can offer longer working distance with high numerical aperture.

(4) Correction Ring
Dry objectives with high Numerical Aperture are susceptible to spherical and other aberrations which can impair resolution and contrast when used with a cover glass whose thickness differs from the specified value. A 11/2 cover glass (0.17mm thick) should be used as standard, however not all 11/2 cover glasses are exactly 0.17mm and many specimens have media between them and the cover glass. The correction ring is used to adjust for these subtle differences to ensure the optimum objective performance.

How to use the correction ring
- Position the ring at 0.17. The thickness of the standard cover glass is 0.17mm.
- Focus the lens on a small artifact in the specimen.
- Rotate the ring very slightly and focus the lens again to check if the image has improved or degraded.
- Repeat the above step to determine if the image is improving or degrading in the direction you are turning the ring.
- If the image has degraded, follow the same procedure in the opposite direction to find the position offering optimum resolving power and contrast.

(5) Retraction Stopper
Some objectives for oil immersion have a retraction stopper. In order to prevent clean slides from being accidentally smeared with immersion oil, the retraction assembly can be engaged by pushing in the front element and twisting it to the right. This will lock the objective in the up position so it will not leave immersion oil on a clean slide as the nosepiece is rotated. Twisting to the left will release the retracted objective for use.

(6) Cover Glass Thickness
For optimum performance, the thickness of the cover glass should be 0.17mm. For example, at NA = 0.95, a 0.01mm difference in thickness reduces image formation by 45% from the ideal image.

(7) Application Markings
DIC: for differential interference contrast
DM: for phase contrast, dark contrast middle type
DL: for phase contrast, dark contrast light type
DLL: for phase contrast, lower contrast type
P: for polarizing
NCG: for use without cover glass

(8) Immersion Oil
After using immersion oil, gently blot the lens dry with lens tissue. Then slightly moisten a piece of lens tissue with petroleum benzene (Naphtha) and clean off all traces of the oil from the immersion objective. Cleaning is essential for water immersion objectives as well; after use, wipe the water off the top lens.
## CFI60 Objectives

<table>
<thead>
<tr>
<th>Type</th>
<th>Use</th>
<th>Model</th>
<th>Immersion</th>
<th>NA</th>
<th>WD (mm)</th>
<th>Cover glass thickness</th>
<th>Correction ring</th>
<th>Spring loaded</th>
<th>Brightfield</th>
<th>Darkfield</th>
<th>DIC</th>
<th>Phase contrast</th>
<th>Polarizing</th>
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*1) Compatible with IMM only  *2) Dedicated for FPI (CFI75 objective)

**Note 1:** Model numbers
**Note 2:** For use with 1.2mm-thick cover glass
**Note 3:** For use with 1.0mm-thick cover glass
**Note 4:** For use with 1.0mm-thick cover glass

**Note 5:** Fluorescence microscopy (UV)

**Note 6:** Universal Plan Fluor

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**Note 1:** Model numbers
**Note 2:** For use with 1.2mm-thick cover glass
**Note 3:** For use with 1.0mm-thick cover glass
**Note 4:** For use with 1.0mm-thick cover glass

**Note 5:** Fluorescence microscopy (UV)
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<th>Use</th>
<th>Model</th>
<th>Immersion</th>
<th>NA</th>
<th>WD (mm)</th>
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<th>Correction ring</th>
<th>Spring loaded</th>
<th>Brightfield</th>
<th>Darkfield</th>
<th>DIC</th>
<th>Phase contrast</th>
<th>Polarizing</th>
<th>Fluorescence</th>
<th>Tri-E PFS</th>
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Note 6: Brightfield/DIC/Fluorescence
- : possible but not recommended
- : suitable
- : recommended for best results
Note 7: Polarizing
- : possible but not recommended
- : suitable
- : retardation measurement is possible with a polarizing microscope
Note 8: Tri-E PFS
- : compatible with PFS