



Objectives for biological microscopes

CFI60



The Ultimate in Optical Performance and System Flexibility

Nikon had two distinct goals in mind when creating its CFI60 optical system for advanced biological research microscopes:

1. To dramatically improve optical performance.
2. To boost overall flexibility of the microscope as a system and increase the performance when various microscope attachments and accessories are used.

To achieve this end, Nikon created a completely new standard for its CFI60 objectives.

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

In these revolutionary optics, both axial and lateral chromatic aberration have been corrected independently in the objective and the tube lens to produce flat images with excellent color reproduction, without the aid of other components.

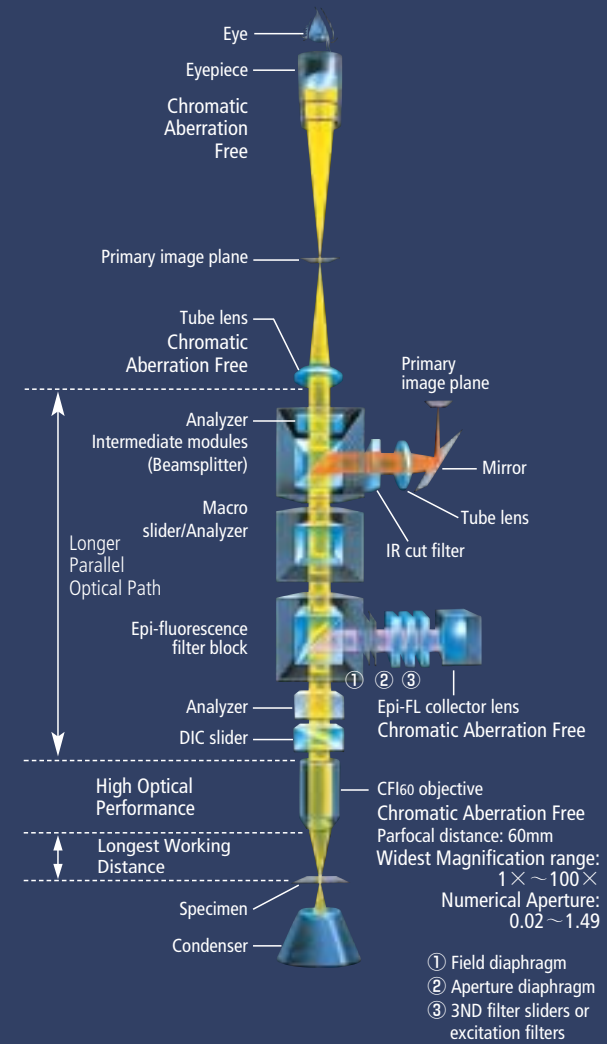
The 200mm tube lens creates a smaller angle between light rays passing through the center and those off axis.

This minimizes shifts between the two light rays when passing through the fluorescence filter cube and DIC prism, dramatically improving contrast during DIC and epi-fluorescence microscopy.

Nikon also designed objectives that curtail auto-fluorescence and flair to create greater contrast during epi-fluorescence observations.

With an array of innovative features, Nikon's CFI60 optical system delivers top-notch performance, enabling their use in increasingly sophisticated biological research.

CFI60 Optical Path (Conceptual Diagram)



CFI60

New Series of Objectives Created with Nikon's Accumulated Optical Technologies

CFI Apochromat TIRF Series

Objectives with world's highest NA of 1.49

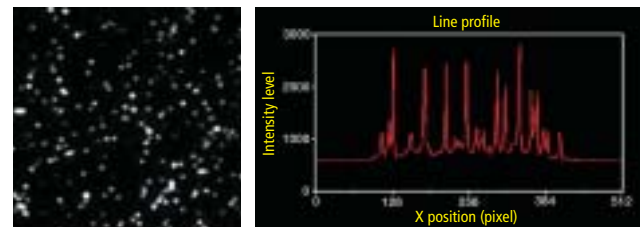
- Because of the unprecedented NA of 1.49—for use with a standard coverslip and immersion oil—these objectives enable the acquisition of bright, high S/N ratio images; so they are suitable for TIRF observation and live cell imaging.
- Both the 60x and 100x lenses utilize the spherical aberration correction 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 correction ring allow acquisition of high-resolution, high S/N ratio images during TIRF observation, episcopic or confocal fluorescence observation as well as Nomarski DIC observation.
- The 100x objective can be optimally applied for laser tweezers microscopy.



CFI Apo TIRF 60x oil, NA 1.49
CFI Apo TIRF 100x oil, NA 1.49

Much higher S/N ratio than a conventional model Sample: Q-Dot

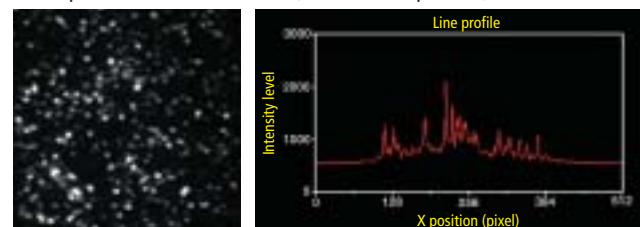
Apo TIRF 100x oil, NA 1.49 (new product)



TIRF image

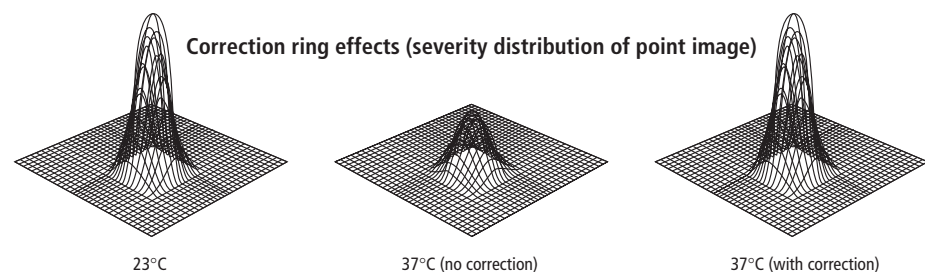
Intensity distribution of fluorescence signal

Plan Apo TIRF 100x oil, NA 1.45 (conventional product)



TIRF image

Intensity distribution of fluorescence signal



Correction ring effects (severity distribution of point image)

23°C

37°C (no correction)

37°C (with correction)

High-sensitivity Apodization Objective for Phase Contrast

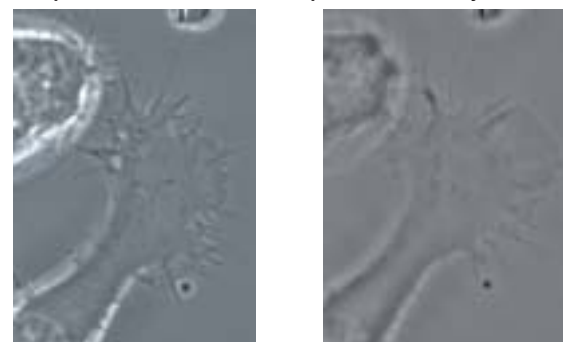
Contrast doubled by reduction in halo

- 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.



CFI Plan Fluor ADH 100x oil, NA 1.30

Comparison with a conventional phase contrast objective lens



NG108-15 cell captured by CFI Plan Fluor ADH 100x oil objective.

The same cell captured by conventional phase contrast objective (CFI Plan Fluor DLL 100x oil).

Images: from The 29th Optics Symposium (2004, Tokyo) 43-46
Cooperation: Dr. Kaoru Kato, Neuroscience Research Institute, The National Institute of Advanced Industrial Science and Technology (AIST)

References: Kaoru Kato, Tatsuro Ohtaki, Motohiro Suzuki (2004) Biophysics Vol 44, No 6, 260-264

CFI Plan Apochromat VC Series

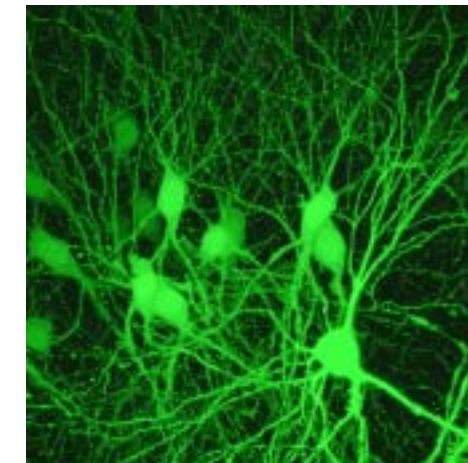
Essential for confocal observation such as DAPI

- 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.



CFI Plan Apo VC 60x oil, NA 1.40
CFI Plan Apo VC 60x WI, NA 1.20
CFI Plan Apo VC 100x oil, NA 1.40

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

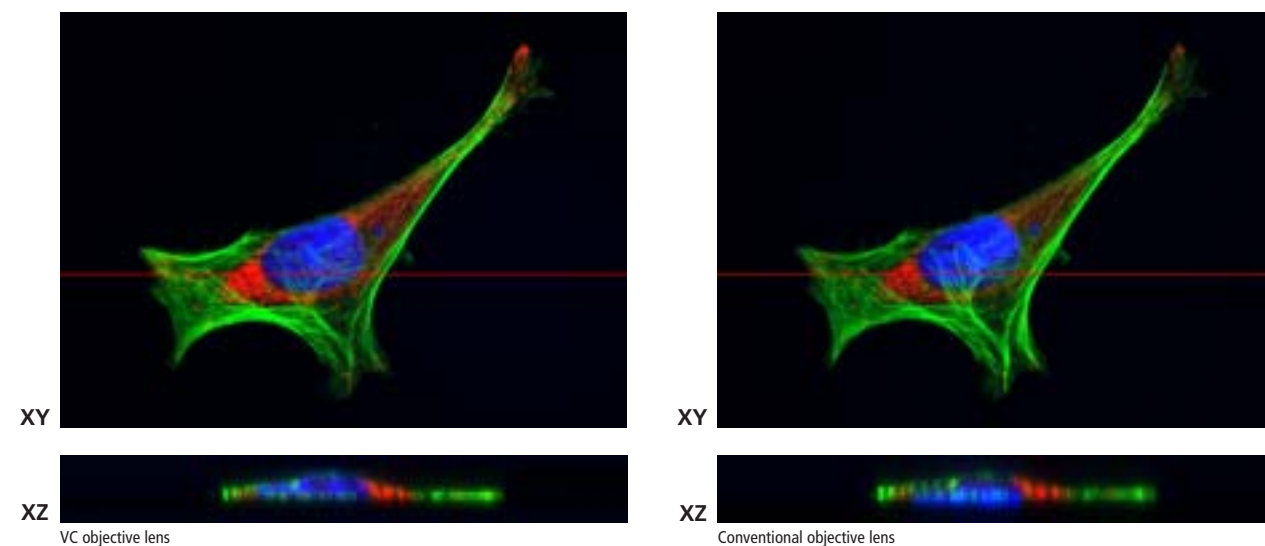


Overlaid consecutive cross-sectional scan within 108µm thickness range of a brain slice with neuronal cells expressing GFP.

Image courtesy of:
Professor Shigeo Okabe and Tatsuya Umeda, Department of Cell Biology, School of Medicine, Tokyo Medical and Dental University

Comparison of conventional lens and VC objective lens

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 Apo VC 100x oil objective lens.

Water-immersion Objective Lens Series

New design for enhanced operability

- Long W.D. 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.



Images courtesy of:
Hiroyuki Hakozaki MS, Ellisman Laboratory, University of California, San Diego, Center for Research in Biological Structure, National Center for Microscopy & Imaging Research (NCMIR)



CFI Plan Fluor 10x W, NA 0.3, W.D. 3.5mm
CFI75 LWD 16x W, NA 0.8, W.D. 3.0mm*
CFI Apo 40x W NIR, NA 0.8, W.D. 3.5mm
CFI Apo 60x W NIR, NA 1.0, W.D. 2.8mm
CFI Plan 100x W, NA 1.1, W.D. 2.5mm

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

Single objective covers a wide range of magnifications

- The 16x objective lens, when combined with FN1 microscope and dedicated magnification module, provides 5.6x, 32x, and 64x magnifications. As it allows observation from a low magnification wide field to a high magnification high resolution field with single objective, the lens is ideal for patch-clamp experiments.

- Ultrawide field of view of 2mm (magnification 5.6x) and wide 45° approach angle make the manipulator control and positioning easy.

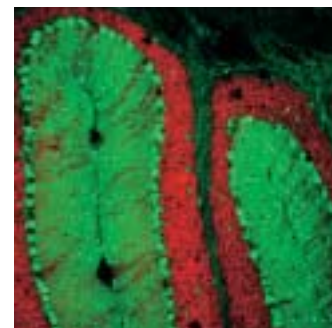


5.6x (magnification 0.35x) 32x (magnification 2x) 64x (magnification 4x)

Images courtesy of:
Dr. Hiroyoshi Miyakawa, Dr. Shigeo Watanabe, Tokyo University of Pharmacy and Life Science

- With excellent IR transmission, this lens is also suitable for IR-DIC observation.

The 16x objective is most effectively used in combination with confocal laser microscopes



Fluorescence image



DIC image

GABA ERGIC neuron (green: GFP) and potassium channel protein (red: YFP) in a cerebellar slice
Images courtesy of:
Dr. Thomas Knöpfel, Team Leader, Laboratory for Neuronal Circuit Dynamics, Brain Science Institute, RIKEN

*16x objective can be used only in combination with a FN1 microscope and single objective holder.

Objectives for brightfield observation



CFI Plan Apochromat Series

This CFI Plan Apochromat series features longer working distances with high Numerical Apertures and is designed to correct all optical aberrations throughout the visible spectrum from violet to red from center to edges across the entire 25mm field of view. Superior image flatness and color reproduction, plus resolving power at the theoretical limit of today's optical technology are also featured.



CFI Plan Fluor Series

Featuring an extra-high transmission rate, especially in the ultraviolet wavelength, and flatness of field comparable to the CFI Plan Achromat series, the CFI Plan Fluor series is designed for fluorescence observation and photomicrography. Because of this improvement in quality, these objectives can function as multi-purpose objectives for brightfield, fluorescence, polarizing, and DIC observations.



CFI Plan Achromat Series

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. These objectives are suitable not only for laboratory work but for photomicrography.



CFI Achromat Series

Correction for chromatic aberration in this series has been dramatically improved and is now at the same level as the CFI Plan Achromat Series. These CFI Achromat objectives were also corrected for spherical aberration and coma and image flatness across the 22mm field of view has been drastically improved. The result: truly exceptional quality for this class of objectives.



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 objective have improved signal-to-noise ratios (S/N) for short wavelengths and have high NA, making the fluorescence images they produce significantly sharper and brighter.

Objectives for Hoffman Modulation Contrast observation



Hoffman Modulation Contrast Series

These objectives have been completely redesigned by Nikon, allowing the contrast direction to be changed using a modulator inside the objective. That direction, once set, is maintained over the entire magnification range from 10X to 40X.

Note: Hoffman Modulation Contrast and HMC are registered trademarks of Modulation Optics Inc.

Objectives for phase contrast observation



CFI Plan Apochromat Series for Phase Contrast

Correction for chromatic aberration has been improved and now extends across the entire visible spectrum to include the violet wavelength. High Numerical Apertures with longer working distances, comprehensive aberration correction, and superior flatness of field of view make Nikon's CFI Plan Apochromat series for phase contrast ideal for the most demanding research projects. Moreover, these objectives can be used for DIC observation.



CFI Plan Fluor Series for Phase Contrast

These objectives are multi-purpose; they can be used for brightfield, fluorescence, phase contrast, or Nomarski DIC observations. They facilitate high-quality fluorescence observation and provide exceptionally detailed resolution of minute structures in phase contrast or DIC observation. 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.



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 Achromat Series for Phase Contrast

Correction for chromatic aberration in this series has been dramatically improved and is now at the same level as the CFI Plan Achromat Series. These objectives now boast performance far outstripping their cost.

Long working distance objectives for phase contrast observation



CFI Plan Fluor ELWD Series for Phase Contrast

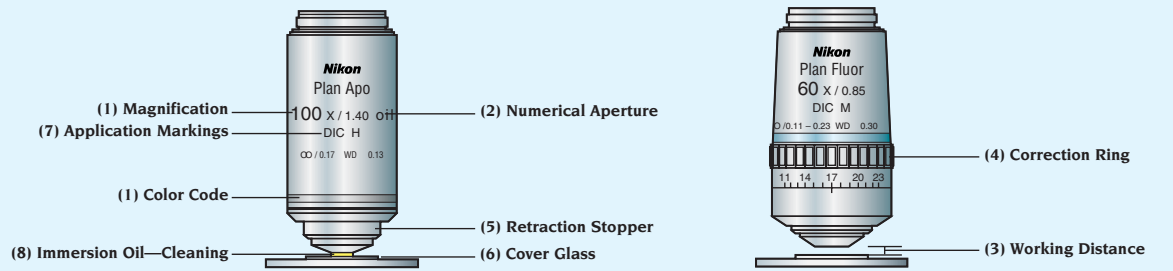
Offering superb flatness of field, high UV transmission rates, and high Numerical Apertures with extra long working distances, these objectives are well suited for fluorescence observations, especially with inverted microscopes. Because of their superior optical design, CFI Plan Fluor ELWD DM objectives can be used universally for all other observation techniques, including brightfield, phase contrast, and Nomarski DIC.

New objectives for apodized phase contrast observation



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. Division activities taking place within a specimen—hitherto often obscured by unwanted halos—can now be observed more clearly.



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:

Mag.	1X	2X	4X	10X	20X	40X	50X	60X	100X
Color code	Black	Gray	Red	Yellow	Green	Light Blue	Light Blue	Cobalt Blue	White

(2) Numerical Aperture (NA)

NA is the most important factor in defining the performance characteristics of an objective.

$$NA = n \sin \theta$$

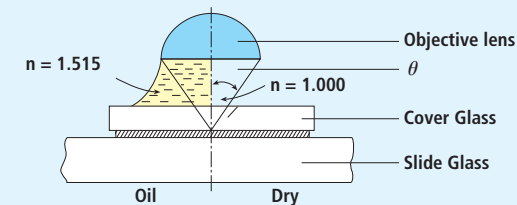
n: the refractive index of the media at d-line (587nm)

For dry objective n=1.000 (air)

For oil objective n=1.515 (oil)

For water objective n=1.333 (water)

θ : Half angle of incident rays to the top lens of the objective



The higher NA, the higher resolving power.

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

$$R = 0.61 \frac{\lambda}{NA}$$

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

The higher NA the brighter image we take.

$$\text{Brightness: } B \propto \left\{ \frac{NA}{\text{Total Magnification}} \right\}^2$$

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

$$DOF = \frac{n \lambda}{2NA^2}$$

(3) Working Distance

Working distance (W.D.) defines the distance between the top lens of the objective and the surface of the cover glass.

CFI60 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 1 1/2 cover glass (0.17mm thick) should be used as standard, however not all 1 1/2 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

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.

NA	Difference in cover glass thickness	
	0.01mm	0.02mm
0.3	100%	100%
0.45	100	100
0.7	98	92
0.85	81	43
0.95	45	29

(7) Application Markings

DIC: for Differential Interference Contrast

DM: Phase contrast, Dark contrast middle type

DL: Phase contrast, Dark contrast light type

DLL: Phase contrast, Lower contrast type

(8) Immersion Oil—cleaning

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.

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. May 2007 ©1998-07 NIKON CORPORATION

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