M287 E 04.11.NF.3

Nikon

ECLIPSE TE2000

Differential Interference Contrast Attachment

Instructions

Thank you for purchasing the Nikon products.
This instruction manual is written for the users of the Nikon Differential Interference Contrast Attachment for the Inverted Microscope ECLIPSE TE2000.
To ensure correct usage, read this manual and the instruction manual supplied with the microscope carefully before operating the instrument.

- It is prohibited to reproduce or transmit this manual in part or whole without Nikon's expressed permission.
- The contents of this manual are subject to change without notice.
- Although every effort has been made to ensure the accuracy of this manual, if you note any points that are unclear or incorrect, contact your nearest Nikon representative.
- Some of the products described in this manual may not be included in the set you have purchased.
- Be sure to read the manuals for any other products (the microscope, etc.) that you are using with this attachment, and always follow all warnings and precautions.
- If the T-A-E motorized DIC analyzer, T-ND6-E sextuple motorized DIC nosepiece, or T-CT-E motorized condenser turret are being used, please refer to the instruction manual supplied with the T-HUBC HUB controller.

Notes on Handling the System

1. Handle the system gently

This system is a precision optical instrument. Handle the system gently, avoiding any physical shocks.
In particular, the optical system used on DIC method must be kept strain-free; handle the objective and condenser carefully so that they are not deformed.

2. Dirty lenses

Do not let dust, fingerprints, etc. get on the lenses. Dirt on the lenses, mirrors, etc. will adversely affect the view of the image. If any of the lenses gets dirty, clean it as described in chapter VI, " Care and Maintenance."

3. Installation location

In order to avoid degraded performance and to prevent malfunctions, take the following requirements into consideration when selecting a location to install the system:

- Install the system in a location with little vibration.
- Avoid installing the system in a location exposed to direct sunlight.
- Avoid installing the system in a dusty location.
- Avoid installing the system in a location subject to high temperatures (40˚C or higher) or high humidity (60% or higher). (Such conditions could allow mold or condensation to form on the lenses and filters.)

4. Specimens

Use glass slides and cover glasses that are free of dirt, scratches, and distortion when preparing specimens.

5. About this DIC attachment

This DIC attachment employs the “Senarmont System,” in which the contrast of the DIC image is varied by the direction of the polarizer.
The DIC prisms are to be installed near the objective and the condenser lens. This attachment requires the correct combination of the objective, condenser lens and the DIC prisms. Read the following notes to choose the correct combination.

5.1 Combination for ∞L / ∞M / ∞H / ∞SS type

For condenser modules (DIC prism for condenser) of the “∞L”, “∞M”, “∞H”, or “∞SS” marking, use the following combinations.

- **DIC prisms for objective :**
  DIC prisms for objective and objectives work in specific pairs. Use the DIC prism for objective that has the same name to the objective.
- **Condenser modules (DIC prisms for condenser) :**
  The condenser module to be used (the DIC prism for condenser) is determined by the N.A. (numerical aperture) of the objective and the type of the condenser lens. (The N.A. of each objective is indicated on the decorative ring of the objective.)

For LWD condenser lens

Objective N.A.	Condenser cassette to be used
N.A. < 0.5	∞ L (black letters on white base)
0.5 ≤ N.A. < 1.0	∞ M (black letters on white base)
1.0 ≤ N.A.	∞ H (black letters on white base)

For ELWD condenser lens

Objective N.A.	Condenser cassette to be used
N.A. < 0.5	∞ L (white letters on red base)

For dry type high N.A. condenser lens

Objective N.A.	Condenser cassette to be used
0.5 ≤ N.A. < 1.0	∞ M (white letters on green base)
1.0 ≤ N.A.	∞ H (white letters on green base)

For water-immersion type high N.A. condenser lens

Objective N.A.	Condenser cassette to be used
0.5 ≤ N.A. < 1.0	∞ M (white letters on blue base)
1.0 ≤ N.A.	∞ H (white letters on blue base)

For oil type high N.A. condenser lens

Objective N.A.	Condenser cassette to be used
1.0 ≤ N.A.	∞ H (black letters on yellow base)
1.0 ≤ N.A.	∞ SS (black letters on yellow base)

- **◪ mark :**
  The DIC prism for objective having the ◪ mark (DIC prism for Plan Fluor 10x), and the DIC prism for condenser having the ◪ mark (LWD condenser ∞L) should be used in pairs.

5.2 Combination for N1 / N2 / NR type

For condenser modules (DIC prism for condenser) of the “N1”, “N2”, or “NR” marking, see the table 1 on the next page and select the objective, DIC prism for objective, and DIC prism for condenser (condenser module) respectively.
Standard combinations, high contrast combinations, and high resolution combinations are listed. Use the appropriate combination.
For ELWD objectives, only one type of condenser lens can be used. See the table 2 on the next page for the combination of ELWD objective, DIC prism for objective, and DIC prism for condenser (condenser module).

Table 1: Combinations of objective and DIC prism

Objective		LWD condenser lens				Dry type high N.A. condenser lens		Oil type high N.A. condenser lens			
		Standard combination		High contrast combination		Standard combination		Standard combination		High resolution combination	
		DIC prism for condenser	DIC prism for objective	DIC prism for condenser	DIC prism for objective	DIC prism for condenser	DIC prism for objective	DIC prism for condenser	DIC prism for objective	DIC prism for condenser	DIC prism for objective
10X	Plan Fluor 10X S Fluor 10X Fluor 10X W	N1 Dry (black letter on white base)	10X								
20X	Plan Fluor 20X Plan Apo 20X S Fluor 20X Plan Fluor 20X MI Fluor 20X W	N2 Dry (black letter on white base)	20X	N1 Dry (black letter on white base)	20X-C	N2 Dry (white letter on green base)	20X	N2 Oil (black letter on yellow base)	20X		
40X	Plan Fluor 40X Plan Apo 40X S Fluor 40X		40X I		40X I-C		40X I		40X I		
	Plan Fluor 40X Oil S Fluor 40X Oil		40X II				40X II		40X II		
	Fluor 40X W Plan Apo 40X Oil		40X III				40X III		40X III		
60X	Plan Apo 60X A Oil Plan Apo 60X Fluor 60X W Plan Apo VC 60X Oil		60X I				60X I		60X I		60X I-R
	Plan Fluor 60X Oil Iris Plan Apo TIRF 60X Oil Plan Apo 60X WI C Plan Apo VC 60X WI Plan Fluor 60X C		60X II				60X II		60X II	NR Oil (black letter on yellow base)	60X II-R
100X	Plan Apo VC 100X Oil		100X I				100X I		100X I		100X I-R
	Plan Fluor 100X Oil Plan Apo 100X Oil Plan Apo TIRF 100X Oil		100X II				100X II		100X II		100X II-R

Table 2: Combinations of ELWD objective and DIC prism

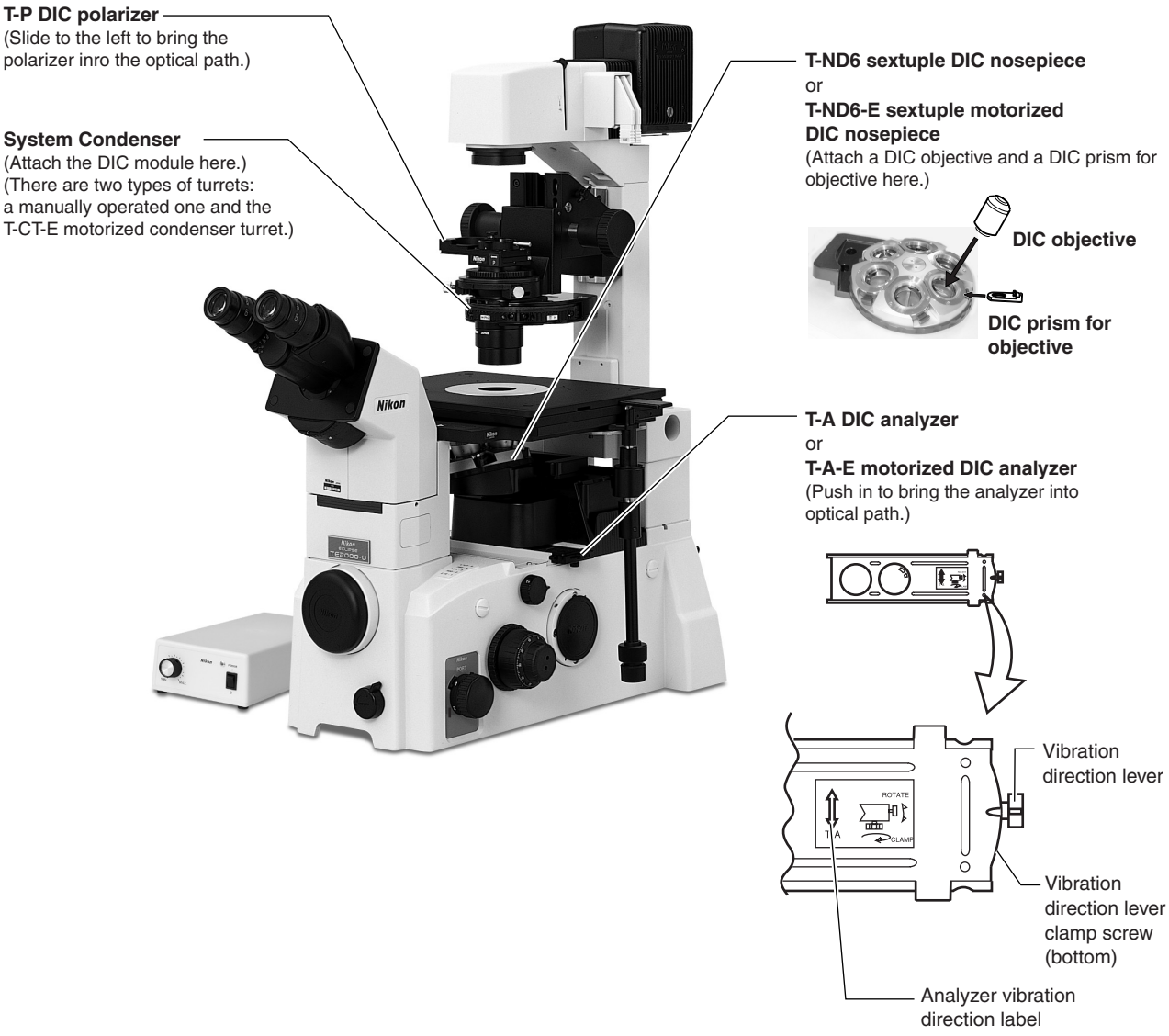
Objective		LWD condenser lens Standard combination	
		DIC prism for condenser	DIC prism for objective
ELWD	PF ELWD 20X C	N1 Dry (black letter on white base)	20X I
	PF ELWD 40X C		40X IV
	PF ELWD 60X C		60X III

CONTENTS

I. Names of Parts

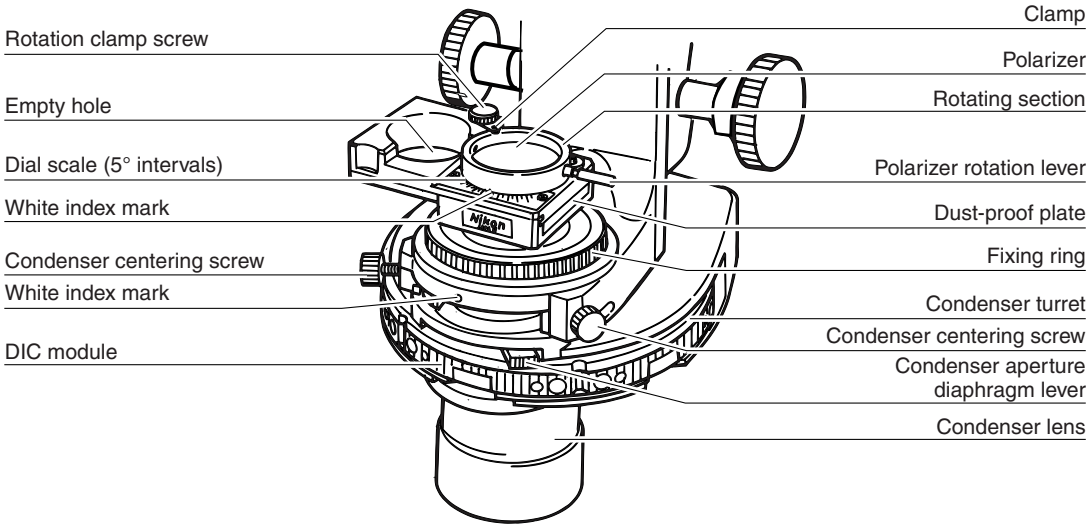
If the system has not yet been assembled, refer to Chapter IV, “Assembly,” first.
For details on the assembly and handling of the microscope, power supply, etc., refer to their respective manuals.

(The Nikon Inverted Microscope ECLIPSE TE2000-U with the DIC attachment mounted is shown below. Some components may not be included in the set that you purchased.)

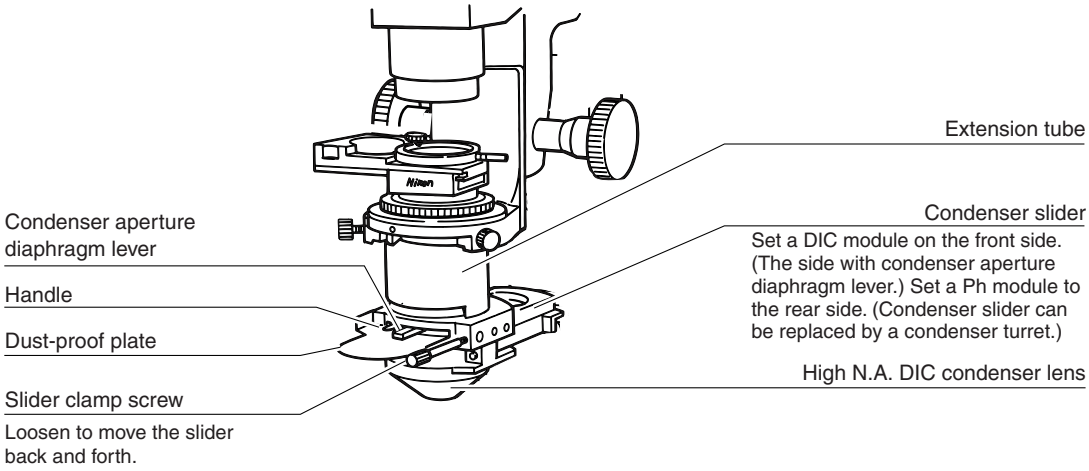


This figure is an illustration of the T-A DIC analyzer. For an illustration of the T-A-E motorized DIC analyzer, please refer to the instruction manual supplied with the T-HUBC HUB controller.

DIC polarizer and system condenser



(When using a high N.A. DIC condenser lens)



II. Microscopy

1 Focus and center the condenser as described in the manual provided with the microscope, and if the T-A-E motorized DIC analyzer, T-ND6-E sextuple motorized DIC nosepiece, or T-CT-E motorized condenser turret are being used, also refer to the instruction manual supplied with the T-HUBC HUB controller.

- Insert the "A" (empty) module into the optical path. (If using the condenser slider, remove the module, or move the slider so that there is nothing in the optical path.)
- Remove the polarizer, the analyzer, and the objective DIC prism from the optical path.
- When using a high N.A. DIC condenser lens:
  In order to focus the field aperture diaphragm image on the specimen surface, and in order to avoid striking the specimen with the condenser lens, move the condenser lens slightly closer than the subject distance for each condenser lens, and then bring the specimen into focus while raising the condenser lens. When using a water-immersion type or oil-immersion type condenser, also check page 9.

Condenser lens	Subject distance
Dry type	5mm
Water-immersion type	4mm
Oil type	1.9mm (including slide glass with a thickness of 1.2mm)

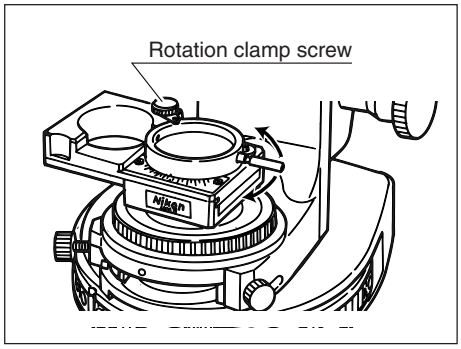
2 DIC microscopy requires that the specimen containers, slide glasses, cover glasses, etc., be largely free of optical distortion. Therefore, a container that will place plastic in the optical path is not suitable for DIC microscopy. In addition, if a direction adjustment is made in the optical system beforehand, it will be possible to observe the DIC image with the best contrast. (See page 15.)

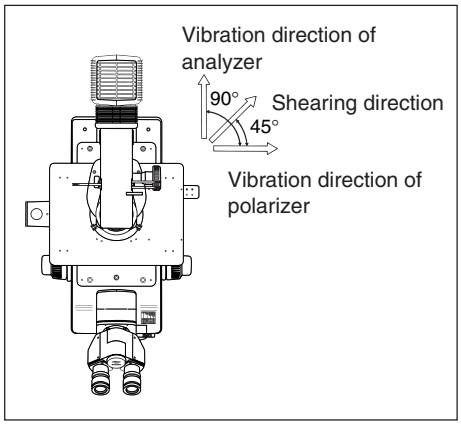
3 Move the DIC 10x objective into the optical path and focus on the specimen. (Stopping down the aperture diaphragm slightly will improve the contrast of the specimen, making it easier to bring it into focus.)

4 Turn the condenser turret and bring the adequate module for the 10x objective into the optical path. (see page 2, 3.)

5 Loosen the rotation clamp screw of the polarizer and then gently rotate the polarizer to change the image contrast.

- The highest contrast is obtained by adjusting the viewfield background to sensitive gray and then bringing the GIF filter into the optical path.
- Because contrast is obtained in the shearing direction (see diagram), adjust the orientation of the specimen until contrast is obtained in the desired direction.





6 Changing the magnification

Ex. 1: DIC LWD 60x N.A. 0.7 and ∞M

(1) Rotate the nosepiece to bring the DIC LWD 60x objective into the optical path.
(2) Because the N.A. of the DIC LWD 60x objective is 0.7, rotate the turret to bring the module labeled "DIC 0.5 - 1.0" into the optical path.
(3) Adjust the contrast by rotating the polarizer.

Ex. 2: Plan Apo 20x and N1 Dry or N2 Dry

(1) Rotate the nosepiece to bring the Plan Apo 20x objective into the optical path.
(2) For the standard and good balance view, bring the "N2 Dry" condenser module into the optical path. For the high contrast view, bring the "N1 Dry" condenser module into the optical path. (See table 1 on page 3.)
(3) Adjust the contrast by rotating the polarizer.

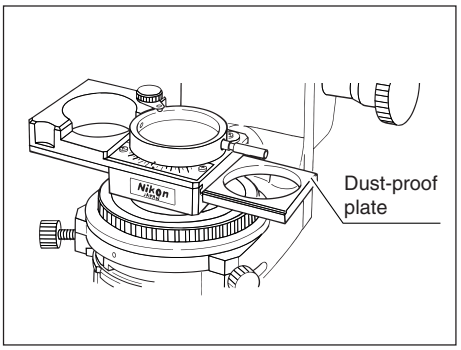
When performing DIC microscopy with high-magnification objectives:

Focus on the specimen using a low-magnification objective (10x). With the specimen in focus, turn the objective refocusing ring to mark the upper limit for the coarse focusing knob. At this point, confirm that (1) the field aperture diaphragm image is correctly focused on the specimen surface, and (2) the same image is correctly centered in the viewfield. If either of these is not true, re-adjust. Once both are true, switch to the high-magnification objective.

When observing a specimen with a high-magnification objective, such as 60x or 100x, first correctly focus on the specimen, and then re-adjust the field aperture diaphragm image on the specimen surface. By doing this, you can easily re-focus on the specimen by focusing on the field aperture diaphragm image.

Color contrast microscopy

Remove the dust-proof plate from the DIC polarizer and insert the lambda plate.
Rotate the polarizer until the background color changes to the sensitive red-violet, a specimen with variations in its refractive index or thickness will show interference colors corresponding to the slope of the variations, producing the highest color contrast.
To observe the image with the correct color temperature, slide the NCB filter into the optical path, and adjust the brightness adjestment dial to match the lamp rating. Use the ND filters to adjust the brightness of the image.



Using a dry-type high N.A. DIC condenser lens

N.A. 0.85, subject distance: 5mm

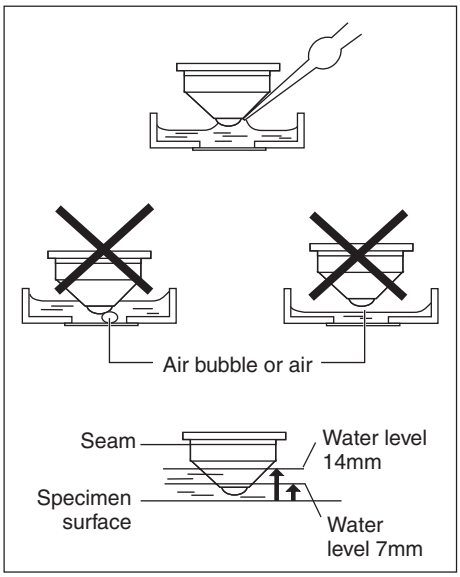
Because the lens tip is cut at a 45° angle to the specimen, a wider work space can be attained.
When using the manipulator, rotate the lens rotation ring so that the cut surface of the lens faces the proper position.

Using a water-immersion type high N.A. DIC condenser lens

N.A. 0.9, subject distance: 4mm

Use with water or culture solution filling the space between the specimen and the lens tip.
This method is suitable when using a cover glass or a culture dish. The lens tip is made of quartz of high purity.

- Water immersion
  Air bubbles at the lens tip can be prevented by letting the water flow down the lens tip. Do not use with an air bubble or air between the lens tip and the specimen.
- Water level limit
  The water level should be 7 to 14mm from the surface of the specimen. In order to prevent water from getting inside the lens, never let the water reach the seam around the lens.
- Container
  High-magnification objectives are generally designed to be used with a cover glass that is 0.17mm thick. (Some objectives have a correction ring.) Therefore, use a specimen container that has glass of a thickness of 0.17mm between the specimen and the lens.



Using an oil type high N.A. DIC condenser lens

N.A. 1.4, subject distance: 1.92mm (slide glass thickness: 1.2mm)

Use with Nikon-specified oil filling the space between the slide glass or cover glass and the lens.
Spread an amount of immersion oil approximately the size of the condenser lens tip on the side of the slide glass or cover glass that faces the condenser. Be careful not to create air bubbles in the oil. The oil should be about 1mm thick. Also apply a small amount of immersion oil on the tip of the oil-immersion objective.
After focusing the field aperture diaphragm image on the specimen surface, tighten the condenser refocusing clamp to mark the lower limit for the condenser. This prevents the tip of the condenser from hitting the specimen and also fixes the field aperture diaphragm image.

Phase Contrast Microscopy and Bright-field Microscopy

Remove the DIC polarizer, analyzer, and objective DIC prism from the optical path before starting microscopy. Because doing so will increase the brightness of the image, use ND filters or the brightness adjestment dial to adjust the brightness.

III. Principles of DIC Microscopy

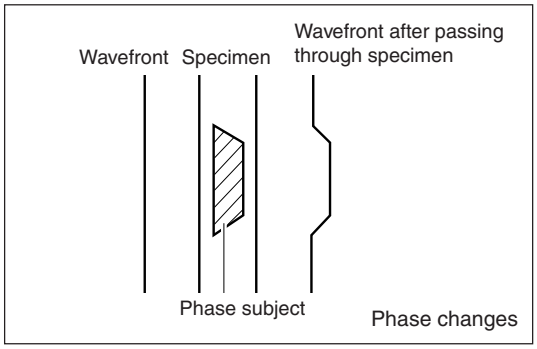
Since the human eye, cameras, and film capture images by recording differences in light intensity and color, they do not register colorless, transparent cells or bacteria. Although dyes can be used to make these transparent subjects visible, the dye itself kills them. The differential interference contrast (DIC) microscope was developed in order to permit the observation of transparent subjects while they are still alive.

The basic structure of the DIC microscope is the same as that of an ordinary microscope, except that it includes polarizing plates and Wollaston prisms*. These optical elements change a transparent subject into different levels of light intensity. In other words, when you look through a DIC microscope, you see even transparent subjects as images consisting of different levels of light intensity. (*Nomarski prisms are also used as a variation of Wollaston prisms.)

The principles of a DIC microscope are discussed below.

Light refracts as it passes through a subject, even if it is a colorless, transparent subject. If the refractive index of a subject is different from that of its surroundings, light that passes through the subject will reach a certain destination point faster (or slower) than light that passes through the surrounding material. In short, when light passes through a subject, it undergoes a phase change (with the phase either getting ahead or being delayed).

A subject whose only effect on light is a phase change is called a "phase subject." See the figure on the right. A wavefront of light that is originally in the same phase, indicated by a single straight line, changes when it passes through a phase subject, so that only the light that passed through the subject traveled faster than the light that did not. This phase change happens when light passes through a microscope specimen, with the light passing through the specimen traveling either faster or slower than the surrounding light.

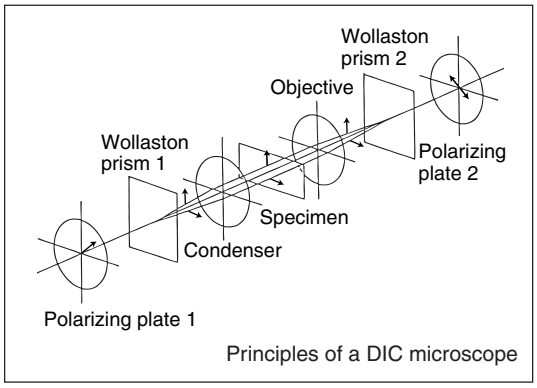


Phase changes

Now look at the figure below. This diagram illustrates the principles of a DIC microscope. In the diagram, the light source is located on the left, so the light travels from left to right. A polarizing plate and a Wollaston prism are each placed on the condenser side and the objective side.

When the light from the light source passes through polarizing plate 1 (the polarizer), it will be polarized (changed to light that vibrates in one direction only). When this polarized light passes through Wollaston prism 1, it is split into two rays that have mutually perpendicular planes of polarization (direction of vibration); the two rays travel at a slight distance from each other.

Since Wollaston prism 1 is on the front focal plane of the condenser, the two rays pass through the specimen in parallel, at a slight distance from each other. The distance between the rays is called the "shear." Since the shear is set below the resolving power of the objective, the specimen does not appear as a double image.



Principles of a DIC microscope

After the two rays pass through the specimen, they are collected by the objective onto the back focal plane of the objective. Wollaston prism 2 is placed here to recombine the two rays back into one. However, the rays do not interfere with each other since they have perpendicular planes of polarization.

Polarizing plate 2 (the analyzer) is placed so that the direction of polarization is perpendicular to that of polarizing plate 1, taking out opposite phase planes in the light. Where the specimen did not change the phase of the light, the two light rays interfere with each other, weakening them and making those areas in the viewfield dark. Where phase objects in the specimen changed the phase of the light, the two light rays do not interfere with each other, so those areas in the viewfield appear bright. This difference in brightness is how the DIC microscope makes phase objects visible.

Comparison of DIC Microscopy and Phase Contrast Microscopy

DIC microscopy and phase contrast microscopy differ in terms of their contrast and image characteristics.
Use the following table as a reference in order to select the method that is best suited to the specimen.

<table>
<tr><th></th><th>Differential interference contrast</th><th>Phase contrast</th></tr>
<tr><td>Image contrast</td><td>The gradient of the optical thickness determines the color or brightness contrast.
Produces a three-dimensional image.</td><td>Differences in the optical thickness of fine structures determines the brightness contrast.</td></tr>
<tr><td>Contrast adjustment</td><td>Adjusted by rotating the polarizer.</td><td>Adjusted by changing objectives.
(Contrast varies according to the type of phase plate and specific absorption of non-diffracted light.)</td></tr>
<tr><td>Image characteristics and detection sensitivity</td><td>Detection sensitivity is high but has directionality.
Contrast is not affected by subject size or phase plate width.
Even specimens with a relatively large phase difference can be observed.
No halo effect.</td><td>Fine objects are easily detected.
Produces halo effect.
Detection sensitivity has no directionality.</td></tr>
<tr><td>Suitable specimens and phase difference tolerance</td><td>Suitable for structures of any size, whether fine or large, even dyed specimens.
Accommodates phase differences of even several wavelengths. Best results if optical thickness gradient is not greater than two wavelengths.
Permits observation even for thicknesses up to 0.5mm. (tissue slices)</td><td>Suitable for subjects with fine structures.
Phase difference:
DL lambda/4 or less
DM lambda/8 or less
BM lambda or less
Best results if thickness is not greater than 10µ.</td></tr>
<tr><td>Depth of focus in photomicrography</td><td>2 to 3 times the phase difference (at high magnification)</td><td>Shallower than for DIC microscopy.</td></tr>
<tr><td rowspan="3">Notes on use</td><td rowspan="2">Match the direction in which you want to add contrast with the shearing direction. (See page 7)
Use distortion-free objectives.</td><td>Center the annular diaphragm and the phase plate accurately.</td></tr>
<tr><td>Use Koehler illumination for best results, and make sure that the light source image is enlarged adequately on the annular diaphragm plane.
Use a culture dish, Petri dish, etc., with glass surfaces that are free of irregularities, smooth and parallel.</td></tr>
<tr><td colspan="2">Protect the surfaces of cover glasses, slide glasses, condensers, and objectives from being dirtied by water, oil, fingerprints, etc. Do not use lens-shaped hole glass, etc.</td></tr>
<tr><td>Brightness</td><td>Slightly brighter than phase contrast microscopy (under monochromatic illumination).</td><td>Approximately as bright as DIC microscopy under white light illumination; slightly darker under monochromatic illumination.</td></tr>
</table>

IV. Assembly

Before assembly, be sure to read all warnings and precautions in the instruction manual for the microscope.
Install the sextuple DIC nosepiece, analyzer, DIC polarizer, and condenser in your microscope by performing steps 1 through 8 below. If you require a very precise vibration direction adjustment, perform step 9 as well.
For details on assembling the microscope, installing the system condenser, etc., refer to the instruction manual provided with the microscope, and if the T-A-E motorized DIC analyzer, T-ND6-E sextuple motorized DIC nosepiece, or T-CT-E motorized condenser turret are being used, also refer to the instruction manual supplied with the T-HUBC HUB controller.

1. Installing the sextuple DIC nosepiece

Place the sextuple DIC nosepiece on the square groove of the microscope's nosepiece mount, sliding the nosepiece toward the rear of the microscope as far as it will go. Tighten the bolts with a hexagonal wrench to secure the nosepiece. (This step is easier to perform if you remove the stage first.)

2. Installing the DIC objectives

Lower the focusing mechanism. Screw the objectives into the revolving nosepiece so that the objectives are in order of increasing power when the revolving nosepiece is rotated in the clockwise direction (when viewed from above).

3. Installing the objective DIC prism

Remove the dummy slider located directly below the DIC objective. Replace the dummy slider with a DIC prism that corresponds with the DIC objective. (Note that the type of DIC prism used depends on the objective.)

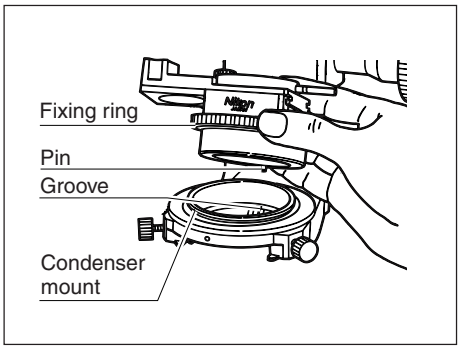
4. Installing the analyzer

Remove the dust-proof slider on the microscope focusing mechanism and insert the analyzer. The analyzer enters the optical path at the second clickstop position. Pulling the slider out to the first clickstop position removes the analyzer from the optical path and places the empty hole in the optical path.
Typically, the analyzer is installed so that the vibration direction adjustment lever is on the right, but it can also be installed with the vibration direction adjustment lever on the left, if you prefer.

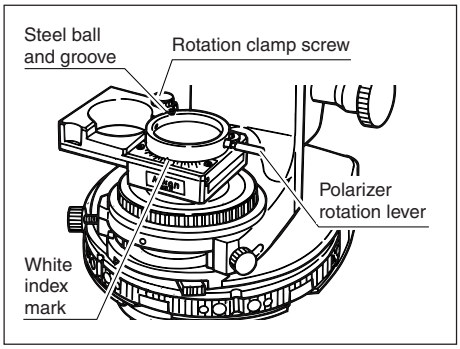
5. Installing the DIC polarizer

1) If the system condenser is attached to the condenser holder, remove it. It is not possible to install the DIC polarizer while the condenser is attached.

2) Mount the DIC polarizer on the condenser mount of the microscope, aligning the DIC polarizer positioning pin with the groove on the condenser mount. Turn the fixing ring to fix the DIC polarizer in place.

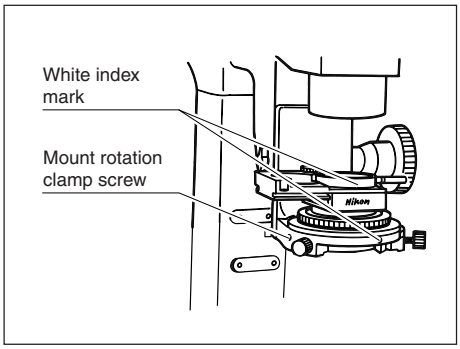


3) Loosen the rotation clamp screw, hold the polarizer rotation lever, and turn the rotating portion so that the white index mark is aligned with the center of the dial scale. (At this point, confirm that the steel ball on the tip of the clamp is in the groove on the rotating section.) Tighten the rotation clamp screw to fix the rotating section in place.



4) Use a hexagonal screwdriver to loosen the condenser mount rotation clamp screw on the left side of the condenser holder. Turn the entire polarizer by hand so that the white index mark that was fixed in place in step 3) is lined up with the white index mark on the condenser holder. Tighten the mount rotation clamp screw so that the polarizer is fixed in place.

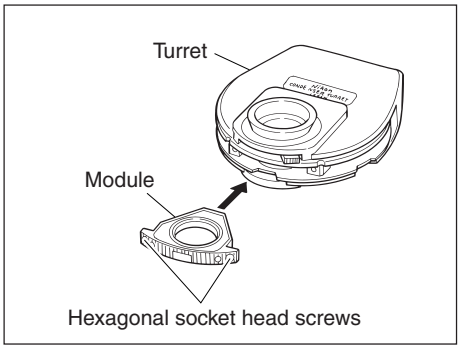
5) The polarizer can now be moved in and out of the optical path by moving the slider.



6. Installing Modules

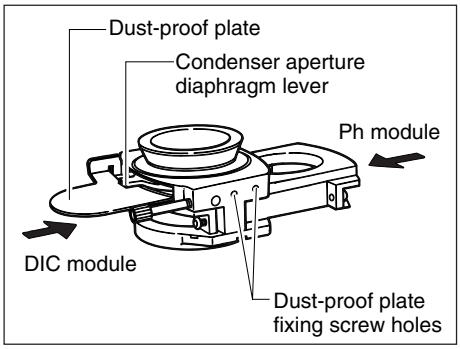
Slide the modules into the condenser turret or the condenser slider and then fix them in place with two hexagonal socket head screws. Note that the modules that can be used are determined by the condenser lens being used. (See page 2, 3.)

- **When using the condenser turret**
  Slide the modules into the turret so that their N.A. or Ph code numbers increase as the turret is rotated in the clockwise direction (when viewed from above). Fix the modules in place with two hexagonal socket head screws.



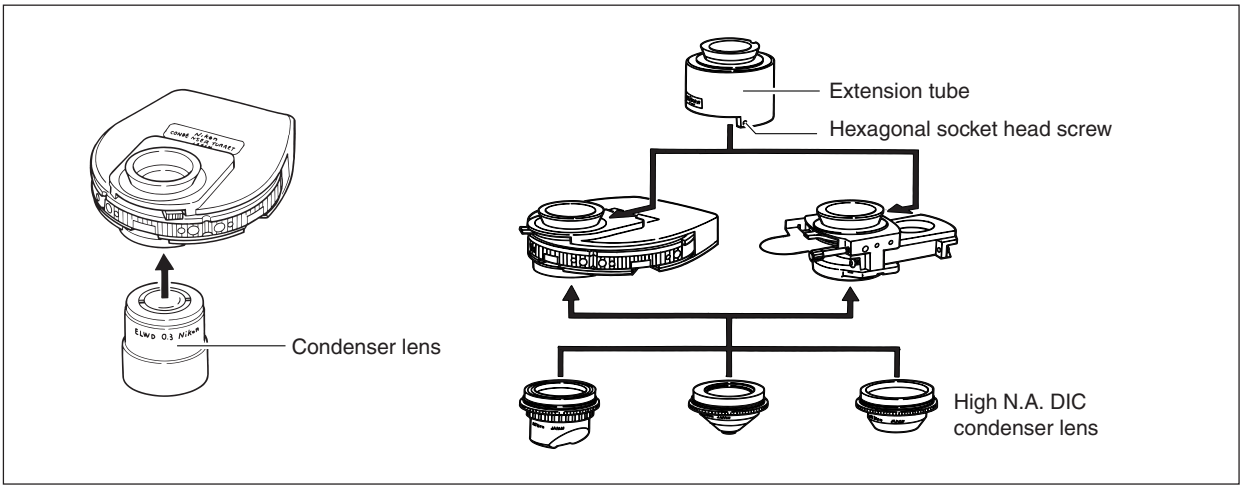
- **When using the condenser slider**
  Set the DIC module in the opening on the side with the condenser aperture diaphragm lever. (Since the inner prism must be used in a certain direction, setting the module on the opposite side makes DIC microscopy impossible.) Instead of a hexagonal socket head screw, screw the slider handle into the left side of the module to fix it in place. Set the Ph module in the opening on the other side.

  The dust-proof plate is fixed in place with two screws. If the screw holes on the opposite side are used, the dust-proof plate can be installed in the reverse position. This is useful if you need more work space in front of the microscope.

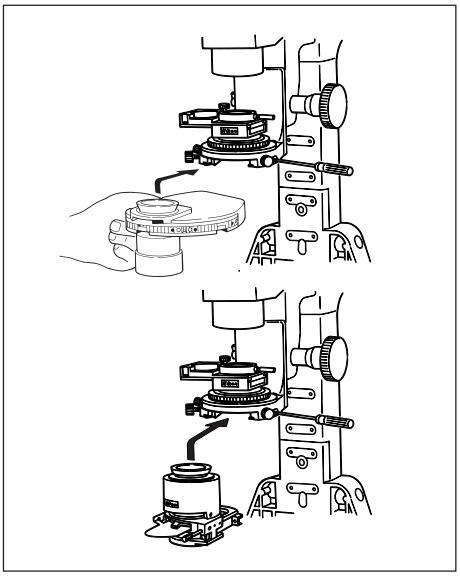


7. Installing the condenser

1) Screw the condenser lens into the condenser turret or the condenser slider. When using a high N.A. DIC condenser lens, mount the extension tube on the round dovetail mount on the top of the turret or slider and then fix it in place with a hexagonal socket head screw.

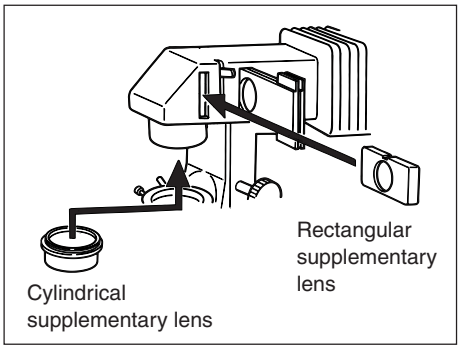


2) Install the completed system condenser on the microscope.
   Loosen the condenser clamp screw (a hexagonal socket head screw) located deep in the right-hand hole on the condenser holder.
   Slide in the system condenser along the dovetail groove, and then fix it in place by tightening the condenser clamp screw. When using the condenser turret, fix the system condenser in place so that the indication on the module above the condenser lens faces the front. When using the condenser slider, fix the system condenser in place so that the indication on the module on the side with the condenser aperture diaphragm lever faces the front.



3) When using an LWD condenser lens or a high N.A. DIC condenser lens, insert the rectangular supplementary lens into the supplementary lens pocket in the diascopic illumination unit.

4) When using a high N.A. DIC condenser lens, screw the cylindrical supplementary lens into the field lens section in the diascopic illumination unit.



8. When using phase contrast microscopy

If you also wish to perform phase contrast microscopy, install the phase contrast objectives and the modules for phase contrast observation that correspond to the condenser lenses to be used, as described in the microscope instruction manual. Before beginning observation, be sure to center the annular diaphragm. (Refer to the microscope instruction manual.)

The assembly procedure is now complete.
If you require a very precise vibration direction adjustment, proceed with step 9.

9. Optical System Direction Adjustment (Adjustment of vibration direction)

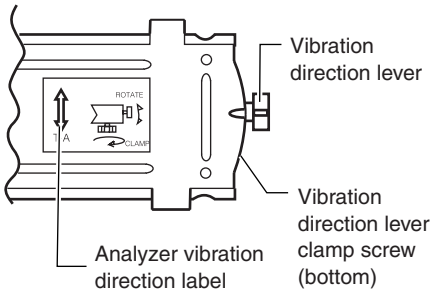
To assure that DIC images can be observed at optimum contrast, the vibration direction of the polarizer and analyzer must be adjusted correctly.

1) Focus and center the system condenser as described in the microscope instruction manual.
   - Bring the A (empty) module into the optical path.
     When using the condenser slider, remove the module that is in the optical path so the slider is empty.
   - Remove the polarizer, analyzer, and objective DIC prism from the optical path.
   - When using a high N.A. DIC condenser:
     In order to focus the field aperture diaphragm image on the specimen surface, and in order to avoid striking the specimen with the condenser lens, move the condenser lens slightly closer than the subject distance for each condenser lens, and then bring the specimen into focus while raising the condenser lens. When using a water-immersion type or oil-immersion type condenser, also check page 9.

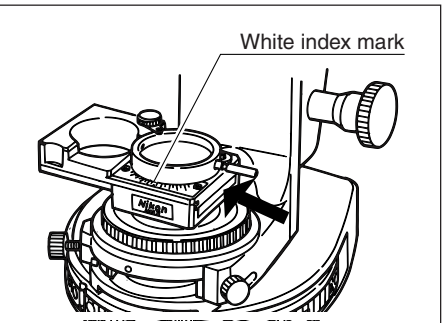
Condenser lens	Subject distance
Dry type	5mm
Water-immersion type	4mm
Oil type	1.9mm (including slide glass with a thickness of 1.2mm)

2) Remove the objective DIC prism that is located directly below the 40x objective.
3) Switch to the 40x objective and bring the specimen into focus.
4) Move the stage so that the specimen moves out of the viewfield. In its place, bring an almost dust-free area into the viewfield. (There must be no dust in the whole viewfield.)
5) Rotate the eyepiece tube turret to the [B] position to bring the Bertrand lens into the optical path so that the objective's exit pupil (the bright circle) can be observed. (If there is no eyepiece tube turret, install a centering telescope instead of an eyepiece and rotate its eyepiece part to focus on the objective's exit pupil.)

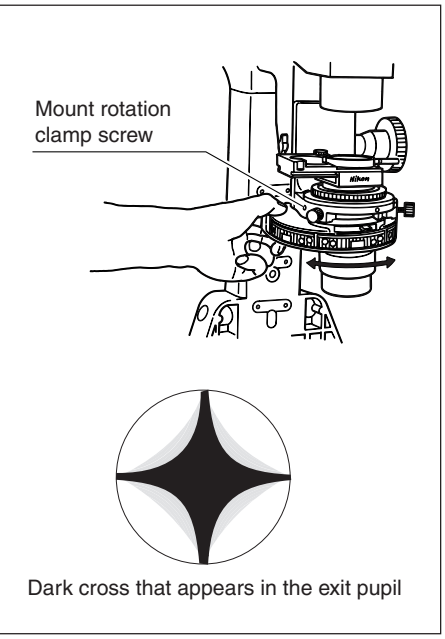
6) Push in the analyzer to the second clickstop so that it is in the optical path. Loosen the clamp screw and align the index on the vibration direction lever with the notch.
(For the locations of the vibration direction lever and vibration direction lever clamp screw on the motorized analyzer, please refer to the instruction manual supplied with the T-HUBC HUB controller.)



7) Push the DIC polarizer slider to the left so that the DIC polarizer is in the optical path.
Confirm that the white index mark on the DIC polarizer is positioned at the center of the dial scale. (If it is not, adjust it as described in step 3) of item 5, “Installing the DIC polarizer,” on page 12.)



8) Loosen the condenser mount rotation clamp screw. Hold the condenser turret and rotate both the condenser and the DIC polarizer at the same time. Tighten the mount rotation clamp screw with a hexagonal screwdriver to fix it at the position where a dark cross* appears in the objective’s exit pupil. Be careful not to accidentally loosen the mount rotation clamp screw during DIC microscopy.
* The dark cross may not be visible with some objectives. In this case, rotate the eyepiece turret to the [◯] position, raise the lamp voltage to the maximum, and then hold the condenser turret and rotate both the condenser and the polarizer at the same time while observing the specimen. When the viewfield is at its darkest, tighten the mount rotation clamp screw.



9) Install the 40x objective’s DIC prism that was removed in step 2). In addition, if the module in the optical path was removed from the condenser slider, install the module as it was originally.

The optical system’s vibration direction has now been properly adjusted.

V. Troubleshooting Tables

If this product is not functioning correctly, consult the following table.

Problem	Cause	Countermeasure
Viewfield vignetting	Optical path switchover dial not in clickstop position.	Rotate to clickstop position.
	The condenser turret is in an intermediate position.	Rotate to clickstop position.
	The objective DIC prism is in an intermediate position.	Move the prism completely into the optical path.
	The analyzer is in an intermediate position.	Set to clickstop position.
	The revolving nosepiece is not installed properly.	Install the revolving nosepiece on the groove.
Contrast not obtained when using the DIC method	The polarizer is not in the optical path.	Insert the polarizer into the optical path.
	The analyzer is not in the optical path.	Insert the analyzer into the optical path.
	Wrong module is selected.	Select a module suited to the condenser lens being used. Select a module suited to the objective's indication.
	The objective DIC prism is not in the optical path.	Move the prism completely into the optical path.
	Wrong combination of objective and objective DIC prism.	Use a prism suited to the objective.
Poor viewing or contrast	Wrong condenser direction.	Determine the crossed Nichols position again.
	Wrong module is selected.	Select a module suited to the condenser lens being used. Select a module suited to the objective's indication.
	Wrong combination of objective and objective DIC prism.	Use a prism suited to the objective.
	There is dirt on the objective, condenser, or specimen.	Gently wipe away the dirt. (Because this is a polarized interference microscope, dirt poses more of a problem than usual.)
	Air bubble in the immersion oil on the lens.	While observing the exit pupil of the objective through the Bertrand lens, turn the revolving nosepiece slightly. If the bubble moves with the nosepiece, the bubble is on the objective side. If the bubble remains stationary, the bubble is on the condenser side. Wipe the immersion oil from the lens and then apply it again.

* If you encounter a problem other than those discussed above, or if the action recommended in the table above fails to correct the problem, refer to the microscope instruction manual.

VI. Care and Maintenance

1. Filter and lens cleaning

Do not let dust, fingerprints, etc. get on the lenses or filters. Dirt on the lenses, filters, etc. will adversely affect the view of the image. If any of the lenses or filters get dirty, clean them as described below.

- Use an air blower to blow away dust. If that does not suffice, brush away the dust with a soft, clean brush, or else wipe it away gently with gauze.
- To remove fingerprints or grease, use a piece of soft, clean cotton cloth, lens tissue, or gauze moistened with absolute alcohol (ethyl alcohol or methyl alcohol). However, do not use the same area of the cloth, etc. to wipe more than once.
- Use petroleum benzine to clean off immersion oil. Wiping with absolute alcohol (ethyl alcohol or methyl alcohol) after the oil has been removed finishes the clean up process.
  If you cannot obtain petroleum benzine, use methyl alcohol. However, because methyl alcohol does not clean as well as petroleum benzine, it will be necessary to wipe the surfaces repeatedly. (Usually, three or four times is sufficient to clean lenses or filters.)
- Use petroleum benzine only to remove immersion oil from objectives; do not use petroleum benzine for cleaning the entrance lens on the eyepiece tube, filters, etc.
- Absolute alcohol and petroleum benzine are both highly flammable. Handle them carefully, especially around open flames and when turning power switches on and off, etc.
- Follow the manufacturer's instructions when handling absolute alcohol and petroleum benzine.

2. Cleaning painted components

Do not use organic solvents such as alcohol, ether, or paint thinner on painted components, plastic components or printed components. Doing so could result in discoloration or in peeling of the printed characters. For persistent dirt, dampen a piece of gauze with neutral detergent and wipe lightly.

3. Storage

Store the system in a dry place where mold is not likely to form.
Store the objectives, eyepieces, filter blocks, etc. in a desiccator or similar container with a drying agent.
Put the vinyl cover over the system after use to protect it from dust.
Before putting on the vinyl cover, turn off the power switches for the microscope and the system used together with the microscope, and wait until the lamphouse has cooled.

4. Regular inspection (charged)

Regular inspection (expenses charged) of this system is recommended to maintain peak performance. Contact your nearest Nikon representative for details about regular inspection.