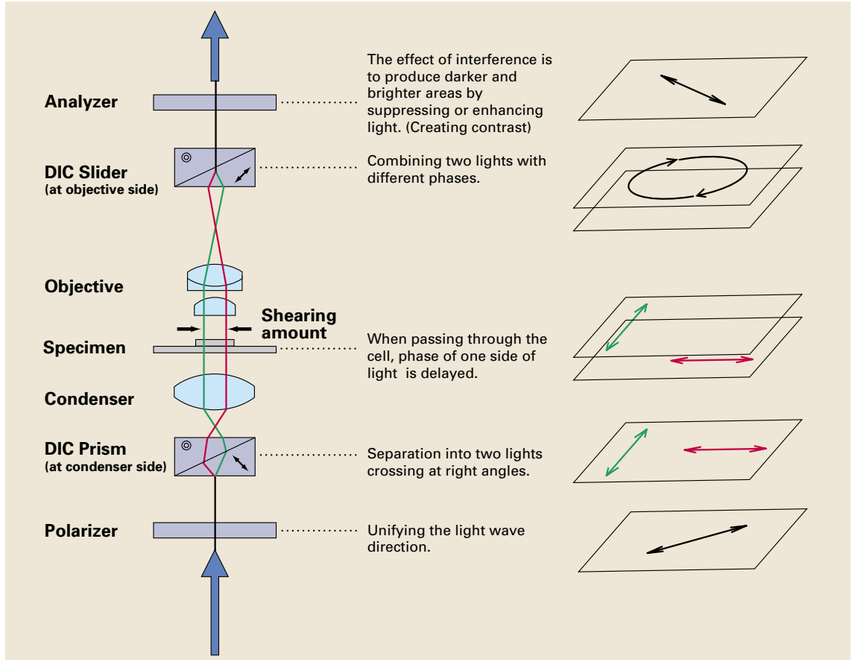
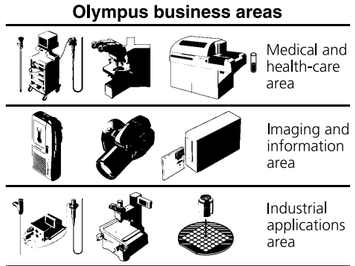
Simple principle of Nomarski DIC microscopy

Nomarski DIC amplifies contrast by using the phase difference which occurs when light passes through material with different refraction values (e.g. a cell) in a particular medium (e.g. water).
The wave direction of light from the microscope light source is unified in a polarizer (condenser side); and when it passes through the condenser side DIC prism, it separates into two phases which cross each other at right angles. The distance of separation is called the shearing amount.
When two such separated lights pass through a medium with different refraction values (e.g. a cell), one of their phase is delayed; and when the two lights are re-composed by DIC slider (the observation side) and analyzer, the interference effect produces the contrast. This is the principle of Nomarski DIC.



Olympus has developed the most suitable DIC prisms for different types of specimen, based on the shearing amount. When DIC contrast is low, the specimen is hard to observe, while high contrast also hinders observation because of excessive glare. Olympus has therefore developed three different types of DIC prisms to ensure clear observation for every kind of specimen.

Olympus business areas



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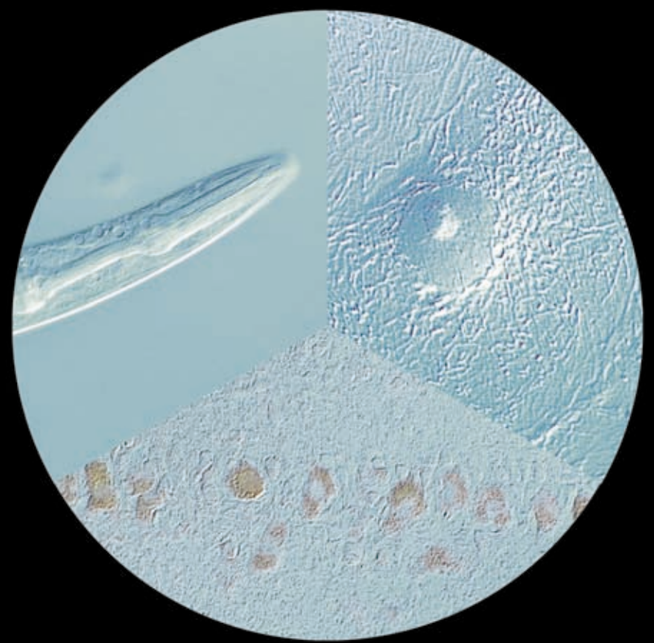
Application of Nomarski DIC microscopy



1 Features of OLYMPUS Nomarski DIC system

1-1 Nomarski DIC slider Lineup

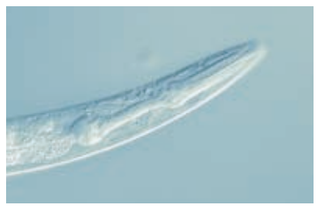
Olympus Nomarski DIC system

Three different kinds of DIC prism are offered as optional components for Olympus biological microscope systems. Their purpose is to provide optimum DIC images according to the wide variety of specimens studied by customers. In addition to the conventional DIC slider for general specimens, two new sliders are now introduced to the lineup, for thick and thin specimens respectively.

Type	For Thick specimen (high contrast specimen)	For Thin specimen (low contrast specimen)	For General specimen
DIC prism	 U-DICTHR	 U-DICTHC	 U-DICT U-DICTS
Indicated color	Blue	Red	White
Appropriate Specimen	Thick specimen Fruit fly embryo *C.elegans* Zebrafish embryo Grasshopper Egg cell Algae Diatoms	Thin specimen Fine structures of cultured cells [on the glass] Low contrast specimen *Cryptosporidium* oocysts	Sliced specimen, tissue slice Rat tissue Mouse tissue Culture cells Pathological tissue
Images	**Crisp, clear image without extra contrast (no glare and noise)**	**Highlights fine structures (which are hard to observe) by amplifying image contrast.**	**Images with good balance between contrast and resolution.**

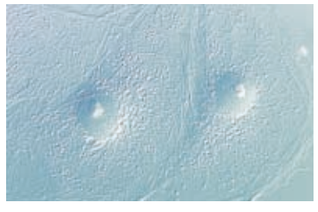
DIC slider for thick specimen : U-DICTHR

In observations of *C.elegans* and Zebra fish embryo, in which the cells are layered structurally, strong contrast hinders image clarity by producing unwanted noise and glare.
The U-DICTHR slider weakens the contrast and presents images with a clear focal plane.



DIC slider for thin specimen : U-DICTHC

Cells that are thinly spread on a glass cover slip, and specimens with low contrast, are much easier to observe if the contrast is emphasized. The U-DICTHC is most appropriate slider for this purpose.



DIC sliders for general specimen : U-DICT U-DICTS

U-DICT/DICTS are sliders whose contrast setting is optimized for observing general specimens such as tissue slices.

1-2 Different DIC sliders, different images

Images provided by DIC sliders are greatly affected by differences in specimen.

1). Thin specimen (Cell culture PtK_2 cells,Thickness: about 2µm)

Objective
PLAPO60X02

For thin specimens such as cultured cells, the U-DICTHC provides clear images with high contrast. This is the most effective way to search the position of low contrast specimens in fluorescence observation.

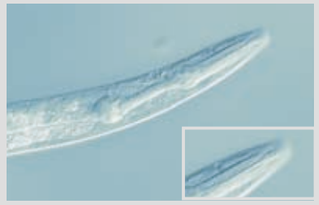
U-DICTHC

U-DICTS

2). Thick specimen (*C.elegans,* Thickness: about 50µm)

For live, thick specimens such as Nematoda (*C.elegans*,etc), the U-DICTHR offers clear, easy-to-observe images with reduced glare (see magnified image in photo, bottom right). It also facilitates observation of the structure on the objective's focal plane, making 3D observation easier, such as injection. In addition, low DIC contrast helps the user to observe stained and non-stained sections simultaneously.

U-DICTHR

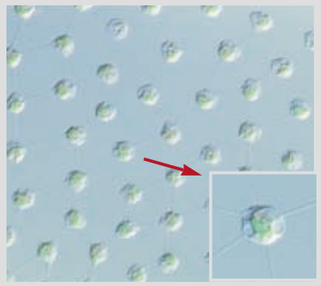


U-DICT

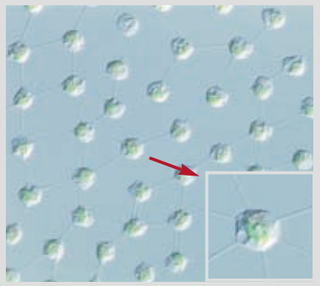
1-3 Differences of image through a DIC slider according to different observed positions

In case of Volvox, thickness is changed depending on the position from which they are observed, so DIC image is changed accordingly. For thick part, the U-DICTHR slider provides a clear image with low glare, while for thin part the U-DICTHC gives more image clarity with high contrast. In the same way, the most suitable slider will be changed according to the position being observed, even in the same specimen.

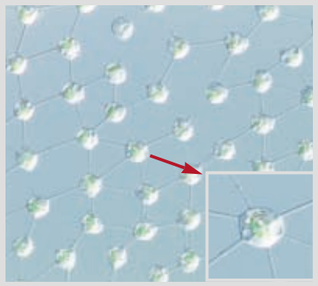
U-DICTHR



U-DICTS



U-DICTHC



Differences in Volvox observation. (See magnified section in photo, bottom right. The cell is in the center.)

	U-DICTHR	U-DICT	U-DICTHC
Image of thin part Connection of cytoplasm (Lined cell structure. Thickness about 2µm)	(Fair) Low contrast, hard to observe	(Good) Well-balanced images of cell connection and cell nucleus	(Very good) Clear image, high contrast
Image of thick part Cell image (Thick part Thickness about 20µm)	(Very good) Interior structures can be clearly observed because glare is reduced		(Fair) It is hard to observe interior structure due to glare

2 Sample photos of Nomarski DIC

2-1 Thick or high contrast specimens

*** Using the U-DICT prism to observe thick specimens, sometimes produces images with excessive glare. In this case, the lower-glare U-DICTHR will be more appropriate.**
*** For low contrast part of thick specimen, other DIC sliders may be more suitable.**

1). Zebrafish embryo (Spinal cord of Zebrafish 24 hours after fertilization), Thickness: about 80µm

The spinal cord nerve cells of zebra fish are composed of multiple cells. For this kind of structure, the U-DICTHR is the most appropriate.

Objective: UPLFL40X

Photo,right: The existence of the primary motor neuron (indicated by arrow) is observed.
Photo,left: Boundary of myotome are observed in a wedge shape and myofibril running vertically are observed inside of myotome. Stripes are seen for each myofibril.

U-DICTHR

2). Fruit fly embryo, Thickness: about 200µm

Since the fruit fly embryo and egg are thick, they have strong glare, the U-DICTHR is the most suitable.

Objective: UPLFL10X PE3.3X

U-DICTHR

In case of using U-DICT

Objective: UPLFL20X PE3.3X

U-DICTHR

In case of using U-DICT

Objective: UPLFL40X PE5X

U-DICTHR

In case of using U-DICT

3). *C.elegans*, Thickness: about 50µm

For observing structure of a *C.elegans*, use of the DICTHR slider provides clear images with reduced glare. In addition, this combination provides images with a clear view on the focal plane, allowing interior mechanisms such as the digestive tract and genital organs to be observed clearly.

Objective: UPLFL40X PE3.3X

U-DICTHR

In case of using U-DICT

4). Hamster egg, Thickness: about 100µm

Combination with the DICTHR allows easy observation of a cell structure, even in the case of round-shaped cell such as hamster egg.

Objective: UPLFL20X PE4X (A part is magnified)

U-DICTHR

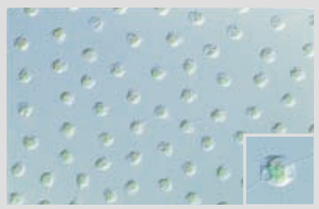
In case of using U-DICT

5). Volvox, Thickness: about 20µm

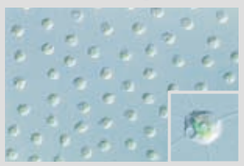
For observation of Volvox, combination with the DICTHR slider provides clear images of the cell structure. However, as shown in the photos below, use of the DICT slider will give clearer images of cytoplasm connection (connecting parts of the cells).

Objective: PLAPO60XO2 PE3.3X

U-DICTHR



In case of using U-DICTS



6). Diatoms, Thickness: about 7µm

Since diatom specimens have high contrast, use of the DICTHR slider will effectively reduce the glare.

Objective: PLAPO60XO2 PE5X

U-DICTHR

In case of using U-DICTS

2-2 Thin or low contrast specimens

1). Cell Culture PtK2 cells, Thickness: about 2µm

Since cell culture specimens tend to be thin, with low contrast, combination with the DICTHC is the most suitable.

Objective
PLAPO60XO2 PE5X

U-DICTHC

In case of using U-DICTS

2). Cell culture N115 cells, Thickness: about 4µm

Objective
UPLFL40X PE4X

U-DICTHC

In case of using U-DICT

3). Cell Culture NG108 cells, Thickness: about 7µm

Objective
UPLFL40X PE3.3X

U-DICTHC

In case of using U-DICT

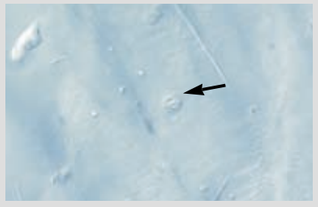
4). *Cryptosporidium* parasite on membrane filter, Thickness: about 5µm

***Cryptosporidium* parasite are observed on the membrane filter. Due to the membrane filter's complex refraction, contrast of DIC image is very low. For such a low contrast specimens, combination with the DICTHC gives the most suitable contrast image observation.**

Objective
UPLAPO100XOI PE4X

Notes when using U-DICTHC
Color unevenness around the peripheral field of view may occur according to specimens. In this case, we recommend using a PE3.3x (or higher) photo eyepiece when taking photos.

U-DICTHC



In case of using U-DICT

2-3 General specimens

U-DICT and U-DICTS are appropriate for both sliced and general specimens.

1). Mouse cerebellum olfactory bulb slice, Thickness: about 20µm

Objective
PLAPIO60XO2 PE2.5X

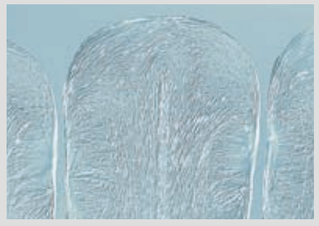
U-DICTS

In case of using U-DICTHR

2). Rabbit taste bud slice, Thickness: about 8µm

Objective
UPLFL20X PE4X

U-DICT

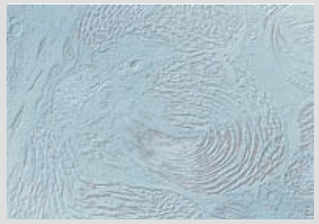


In case of using U-DICTHR

3). Water lizard testis slice, Thickness: about 3µm

Objective
UPLFL40X PE3.3X

U-DICT



In case of using U-DICTHR

In appreciation
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Department of Physiology,Tokyo Woman's Medical University School of Medicine ····· (Page 2 & 4 C-elegans)

Hisayoshi Nozaki, Ph.D.
Department of Biological Sciences, Graduate School of Science, The University of Tokyo ····· (Page 2 & 4 Vorvox)

Hitoshi Okamoto, M.D., Ph.D.
Laboratory Head, Lab. for Developmental Gene Regulation, Developmental Brain Slice Group,
Brain Slice Institute, RIKEN (The Institute of physical and Chemical research) ····· (Page 3 Zebra fish embryo)

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Department of Biological Sciences, Tokyo Metropolitan University ····· (Page 3 Fruit fly)

Teiichi Furuichi, Ph.D.
Laboratory Head, Laboratory of Molecular Neurogenesis, Brain Slice Institute,
RIKEN (The Institute of physical and Chemical research) ····· (Page 6 Mouse cerebellum slice)

Terumasa Sakamoto
Water quality Center, Water Works Bureau of KANAGAWA ····· (Page 5 Cryptosporidium)