The Carl Zeiss Nomarski DIC System

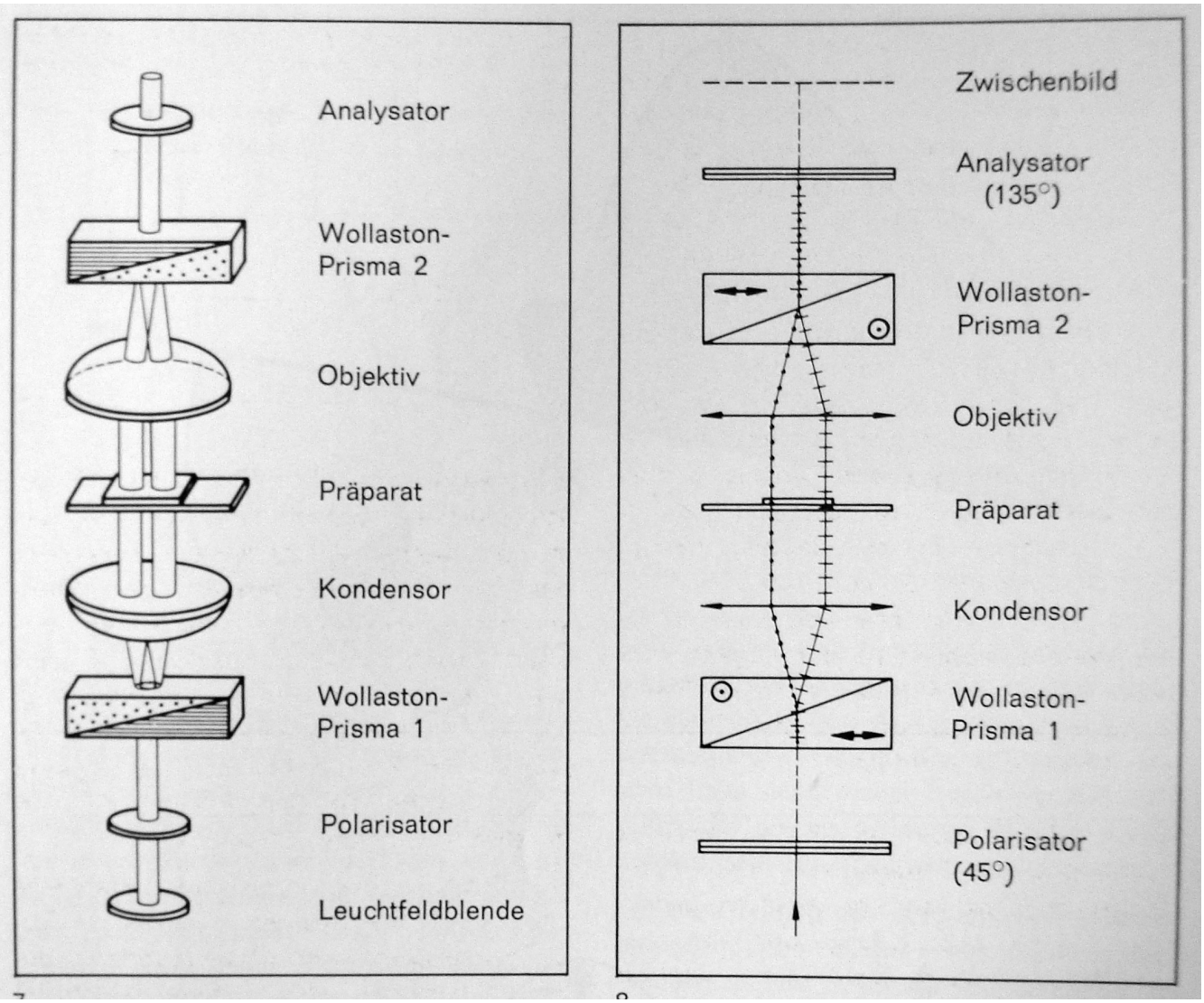
Every so often an article appears dealing with the ZEISS differential interference contrast (DIC) system, in particular, the condensers used. Since its introduction in 1965 the Zeiss DIC sytem underwent several upgrades and I would like to clarify the somewhat confusing situation as best as I can based on the original Zeiss information I have on hand. Unfortunately I do not possess a complete working unit, therefore I cannot test certain procedures myself. However, I would like to point out from the very beginning that unorthodox combinations of DIC components i.e. condenser, objectives, DIC prisms etc. may produce effects similar to DIC but which are in reality only three-dimensional effects like inclined illumination and may lead the inexperienced microscopist to assume he is working with DIC. How to distinguish the one from the other will be explained later on.

Like the first traditional contrast system, the Phase Contrast (after Zernike), the *Differential Interference Contrast* (after Nomarski) is based on the phase shift light acquires when passing through an unstained transparent object. In the case of Nomarski DIC two adjacent beams produced by the first prism are retarded slightly differently as they pass through different locations of the object and interfere once they are recombined by the second prism, thus creating the contrast.

While phase contrast is affected by the entire thickness of the object, DIC is limited to a narrow plane of focus due to its high numerical aperture, resulting also in high resolution. Phase contrast works with a much lower numerical aperture caused by the phase rings with concomitant lower resolution and great depth of field. It also suffers from the “halo” effect. Furthermore, phase contrast is non-directional while DIC is azimuthal with the optimal effect at 45 degrees (a rotating stage is therefore recommended). All this means that objects seen in phase contrast are obscured by what lies above and below the focal plane, while DIC yields a clear image of the object in the focal plane unobstructed by what lies above or below due to the small depth of field.

The principle of Nomarski Differential Interference Contrast:
Please, refer to the following schematic
(Translated from Zeiss Informationen #70/1968 Dr. W. Lang: Differential Interferenzkontrast Mikroskopie nach Nomarski)
“The right-hand schematic shows the respective vibration directions of the polarized light bundles . The polarizer is so oriented that the light exiting the field diaphragm is linear polarized, the vibration plane is tilted 45° to the plane of the drawing.
The lower part of the Wollaston prism 1 splits the incoming polarized wave in two polarized part-waves. The dotted line indicates waves vibrating vertically to the drawing surface, the hatched line waves vibrating parallel to the drawing surface..Both part-waves pass though the specimen parallel and very close together.
After the specimen plane the light beams are reunited by the Wollaston prism 2 and the objective. Note that the condenser with Wollaston prism 1 and the objective with Wollaston prism 2 are functionally connected. The analyzer is so oriented as to form an angle of 45° with the vibration plane of the incoming wave, thus both waves interfere with the same intensity. The interference image is formed in the intermediate image plane in the well known way and enlarged by the eyepiece.”

This principle explanation presupposes that the light-source side focal plane of the condenser and the image side focal plane of the objective are accessible. Higher power objectives' rear focal plane is, however, usually below the “screw in” plane and therefore, inaccessible. This is where *Nomarski's modified Wollaston prism* comes in. It is so designed that the point of interference lies *outside (below)* the prism. As the distance object to intermediate image is constant, the prism can be designed to be located in this accessible plane, hence one prism (prism slider) will serve all objectives 16x, 40x and 100x. Due to mechanical differences, the large research microscopes Universal, Photomicroscope, and Ultraphot require a different slider. II, Standard GfL, RA, and WL use slider III.



(Leuchtfeldblende field diaphragm, *Zwischenbild* intermediate image, *Präparat* Specimen/Object)

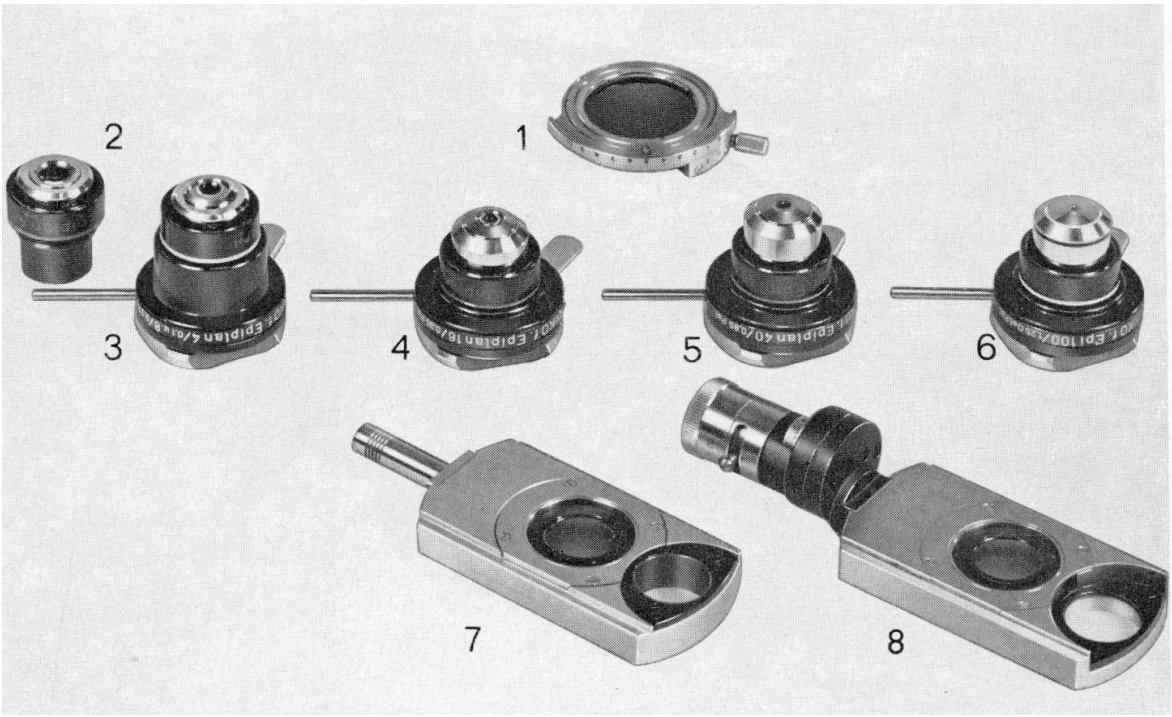
Please note that in this schematic, representing the DIC microscope after *Smith,* the points of interference are located *inside* the Wollaston prisms and in the focal points of condenser and objective. The prisms in the *Nomarski* system are modified so that the point of interference lies *below* the prism 2.

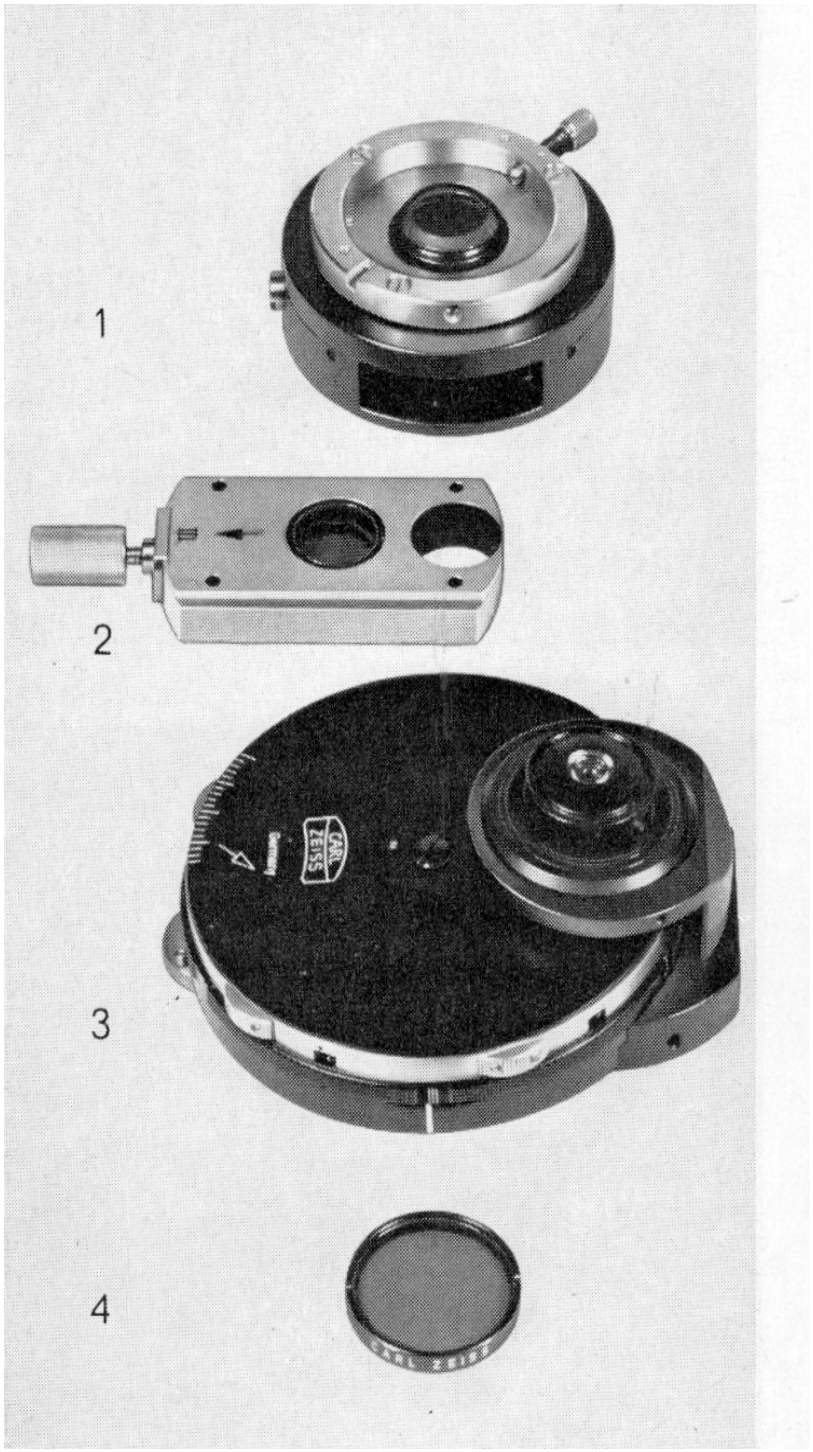
Zeiss specifies exactly which combination of condenser, objectives and prisms to use. Correct and optimal working of DIC depends on the critical interference taking place exactly in the designated plane of the objective used. Zeiss objectives have over time been upgraded and improved without changing the official designation. A Planachromat 40/0.65 of vintage 1960 may not be exactly like a Planachromat 40/0.65 of vintage 1980 as regards its focal planes and as individual serial numbers or the date of manufacture are usually not available to the user, this may pose a problem in certain cases.

Before I start listing the various systems, let me state that a prerequisite to all DIC systems are crossed polarizers. The polarizer below the condenser must be oriented in the E-W direction. The analyzer is usually fixed in its slider, the front lenses of more recent condensers are strainfree and marked in red. All Zeiss DIC systems for transmitted light are specifically designed for either the “Standard” line of microscopes, i.e. GfL , RA or WL, or the large research microscopes with “Tube Head” i.e. Universal, Photomicroscope or Ultraphot. The prisms in the DIC condenser have to be oriented accordingly (see arrow in illustration). This applies particularly to the latest issue with individual small prisms for the objectives. So an old DIC condenser will not work with the new system and a new condenser with the old large sliders. Tough for the “collector” who proudly acquired a mismatched set.

Another point the microscopist using DIC ought to consider is that while phase contrast is non-directional, DIC is azimuthal. Hence a rotating stage is of advantage in order to examine the specimen in all aspects.

One more remark: Zeiss has developed an entirely different system for epi-illumination which uses only one prism in a special rotatable adapter ring for epi-objectives for use with epi-condensers. Epi DIC is rarely referred to, so I shall not describe it here.





Zeiss' first issue of the Nomarski DIC system for transmitted light for objectives 16x, 40x, and 100x*:

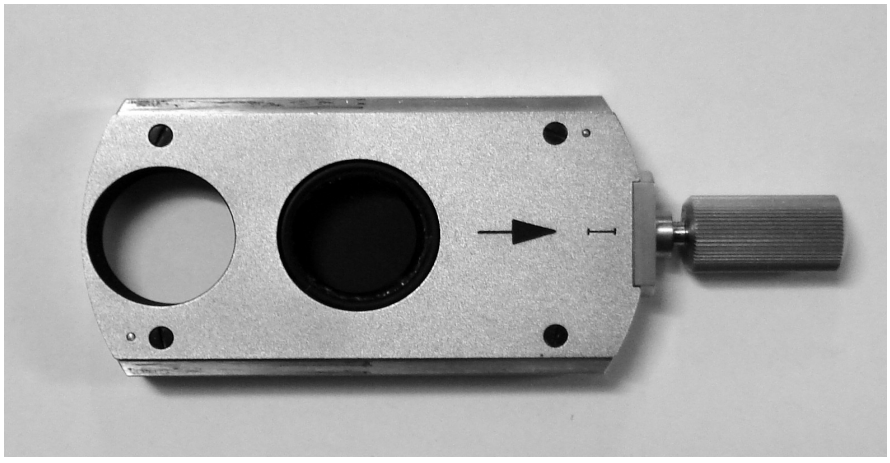
1 Intermediate tube for Standard GfL, and WL
2 Large slider III 47 44 33
3 DIC/Ph condenser 46 52 79
4 Polarizing filter 32mm dia.

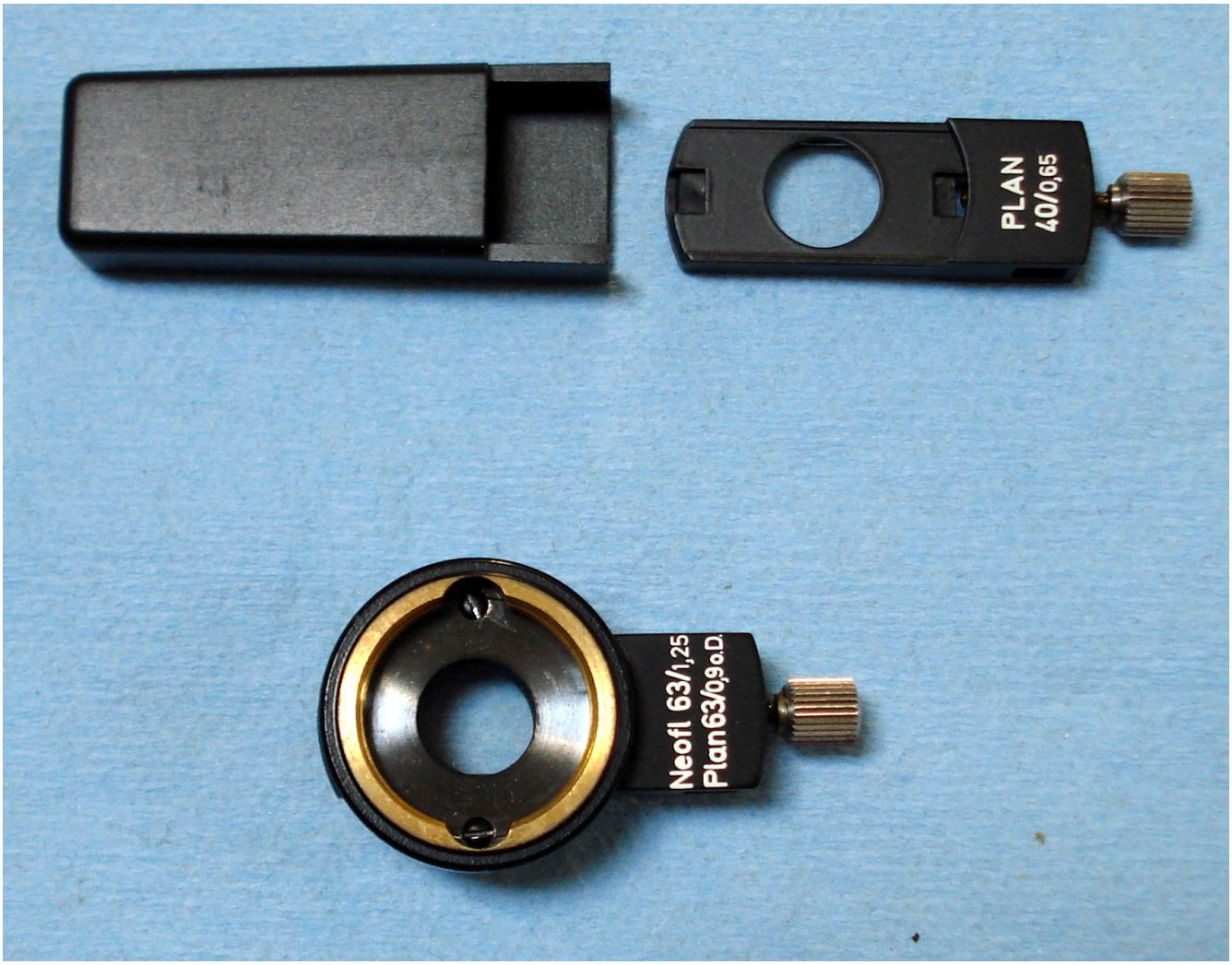
* It is quietly assumed that *Planachromats* are meant

(Photo courtesy Michael Wolfson)

An old Zeiss DIC condenser marked **Inko** and with slanted arrow, possibly for the puzzling large slider I which crops up every so often and for which I could not find any reference at all except a note in my old 1970 pricelist: **46 52 79 "first issue"** (condenser)
47 44 30 slider I. The same pricelist lists : 46 52 84 achromatic-aplanatic Inko-codenser 1.4 Z for DIC, phase-contrast and brightfield, including front lens 0.63 (for slider II and III)

My own useless slider I





(Photo by author)

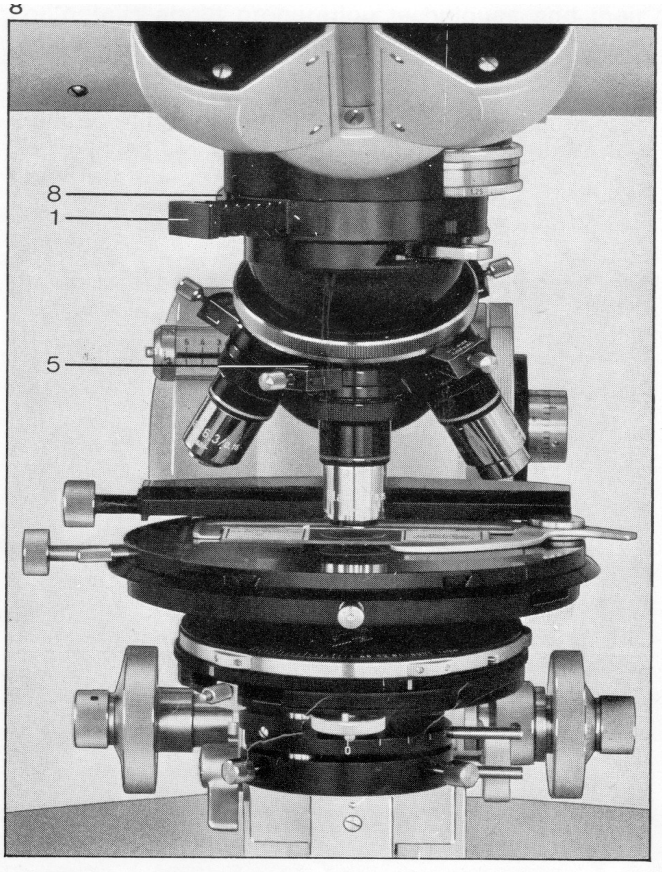
Parts of the latest Zeiss DIC system:

Above - a slider with its protective sheath

Below - another slider in the intermediate (adapter) ring 47 44 65 required for each objective. A special “empty” ring with a compensating lens 47 44 66 adjusts the non DIC objectives for parfocality on the revolving nosepiece to the extended length.

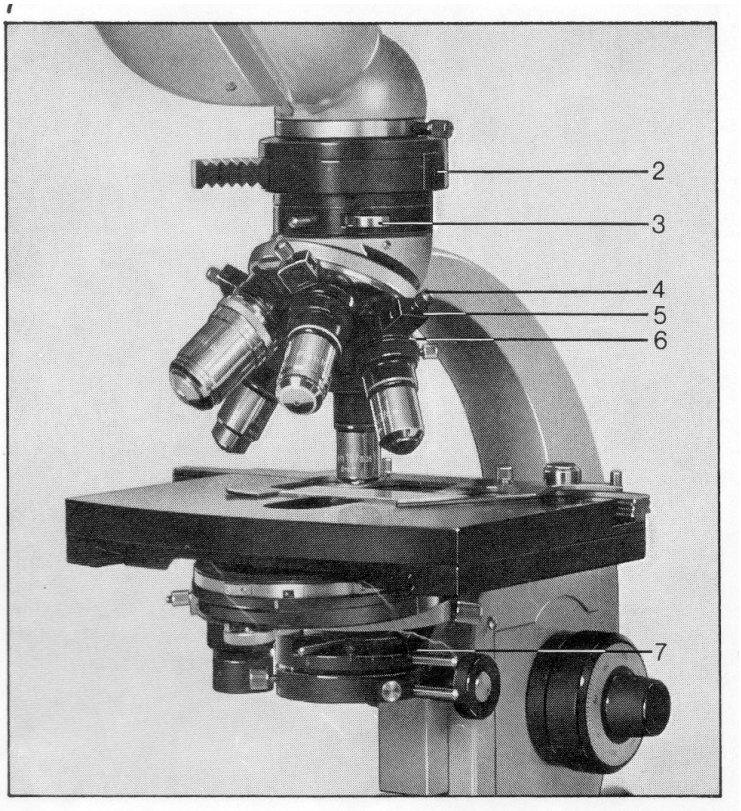
Note that the intermediate ring 47 44 65 is itself adjustable so that the slider is oriented correctly when the unit is screwed on the nosepiece in the working position. A small screwdriver is necessary. The procedure is a bit tricky.

This DIC system was on the market until the Standard line of microscopes was replaced by the **Axio**-Line which is non-compatible with Standard parts and accessories.



Last version of Zeiss DIC equipment on Photomicroscope.

1 = analyzer slider
5 = small prism slider

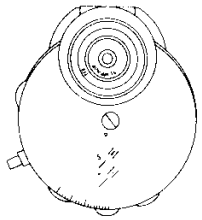


The same equipment shown on Standard RA
2 = analyzer in intermediate tube
3 = optional red 1st order plate
4 = small knob on prism slider to move prism for effect
5 = prism slider
6 = intermediate ring for slider
7 = polarizer (under condenser)

A red 1st order plate can be used for special colour effects.

Identification of Individal Parts

DIC condenser



Achromatic-aplanatic DIC condenser V Z, N.A. 1.4 with brightfield portion and annular phase contrast diaphragms Ph 2 and Ph 3	46 52 85
Achromatic-aplanatic DIC condenser IV Z/7, N.A. 0.63, for up to 7mm back focal lengths, with brightfield portion and annular phase contrast diaphragms Ph 2 and Ph 3	46 52 73
Achromatic-aplanatic condenser 0.32-1.4 Pol Z	46 52 67
Condenser head 0.63 Pol	46 52 65
Condenser head 1.4 Pol	46 52 68

Objective adapter rings, DIC prism slides for objectives

Objective		W.D. mm	DIC Slider	Intermediate Ring
46 03 10	Planach. 6.3/0.16	4.9	47 45 31	47 44 65
46 05 10	Planach. 16/0.35	2.8	47 45 51	47 44 65
46 15 25	Plan-Neo. 16/0.5	0.15	47 45 55	47 44 65
46 16 25	Plan-Neo. 25/0.8	0.3	47 45 60	47 44 65
46 17 02	Ach. 40/0.75W	1.6	43 45 01	47 44 65
46 07 10	Planach. 40/0.65	0.7	47 45 71	47 44 65
46 07 15	LD Planach. 40/0.6 corr.	1.5	47 45 64	47 44 65
46 17 25	Plan-Neo 40/0.9	0.13	47 45 79	47 44 65
46 18 20-9904	Neo. 63/1.25 oil	0.5	43 45 00	47 44 65
46 18 40	Planapo. 63/1.4 oil	0.9	47 45 81	47 44 65
46 19 10	Planach. 100/1.25 oil	0.9	47 45 91	47 44 65

To maintain parfocality for objectives on revolving nosepiece that are not used for DIC :
Intermediate (adapter) ring with compensating lens: 47 44 66

(Photo by author)

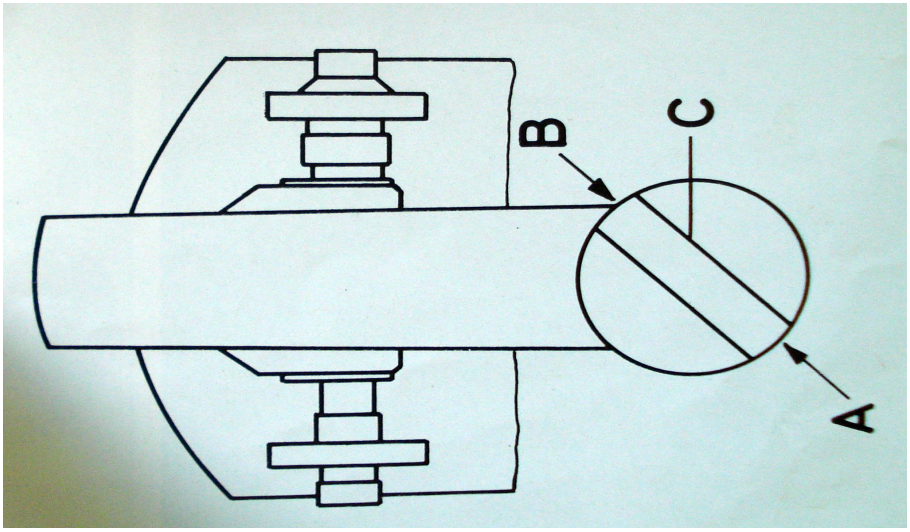
Zeiss DIC / Ph condenser with “red” strainfree optics for polarized light. The suffix -9901 merely indicates a later version of the same condenser with minor design changes.

Note the special markings:

S setting for **S**tandard GfL, RA and WL microscopes (see next illustration)
T setting for microscopes with **T**ubehead i.e. Universal, Photomicroscope and Ultraphot.

A new condenser when bought comes provided with a special tool for orienting the prisms. If you don't have this tool you will have to find a way to rotate the prism into the correct position.

Underside of condenser 46 52 85-9901 with cover removed, showing the condenser prism 43 44 94 for objectives 16x and lower. Arrow indicates the setting for **T :** square mark opposite white index. (Photo by author).



Insertion direction of small DIC slides on objectives: A = for **T**ubehead, B = for **S**tandard microscopes, C = direction of slots for DIC prisms (45°).

Achromatic-aplanatic
DIC phase contrast brightfield condenser
aperture 1.4 (46 52 85)

Condenser positions

Position I DIC prism for Planachromat 6.3/0.16 (incomplete illumination of field of view) Planachromat 16/0.35	Position II DIC prism for LD-Planachromat 40/0.60 corr. Planachromat 40/0.65 Planapochromat 63/1.40 oil Planachromat 100/1.25 oil (all apertures can be fully illuminated)

Position J for brightfield only
Position 2 or 3 for phase contrast with objectives designated Ph 2 or Ph 3.
Position III for special prisms.

“Position” refers to the white indicator line on the side of the condenser's rotating selector. Note that there is no “darkfield” position on this condenser.

Achromatic aplanatic
DIC phase contrast brightfield condenser IV Z/7
aperture 0.63 (46 52 73)
Long focal intercept (distance between front lens and specimen):
7 mm in air, 11 mm in glass, to illuminate thick specimens or specimens in culture vessels.

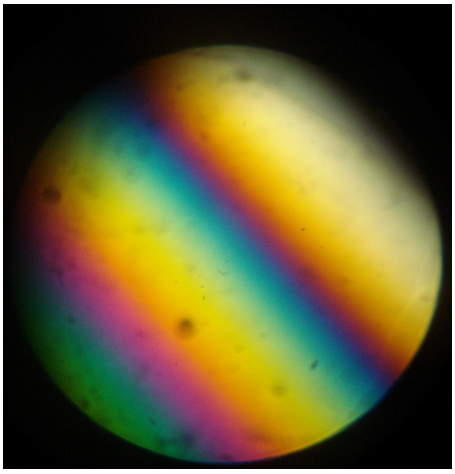
Condenser positions

Position I DIC prism for Planachromat 6.3/0.16 Planachromat 16/0.35 (all fields of view can be fully illuminated)	Position II DIC prism for LD-Planachromat 40/0.60 corr. Planachromat 40/0.65 Planapochromat 63/1.40 oil Planachromat 100/1.25 oil (apertures up to max. 0.63 can be illuminated)

Position J for brightfield only
Position 2 or 3 for phase contrast with objectives designated Ph 2 or Ph 3.
Position III for special prisms.

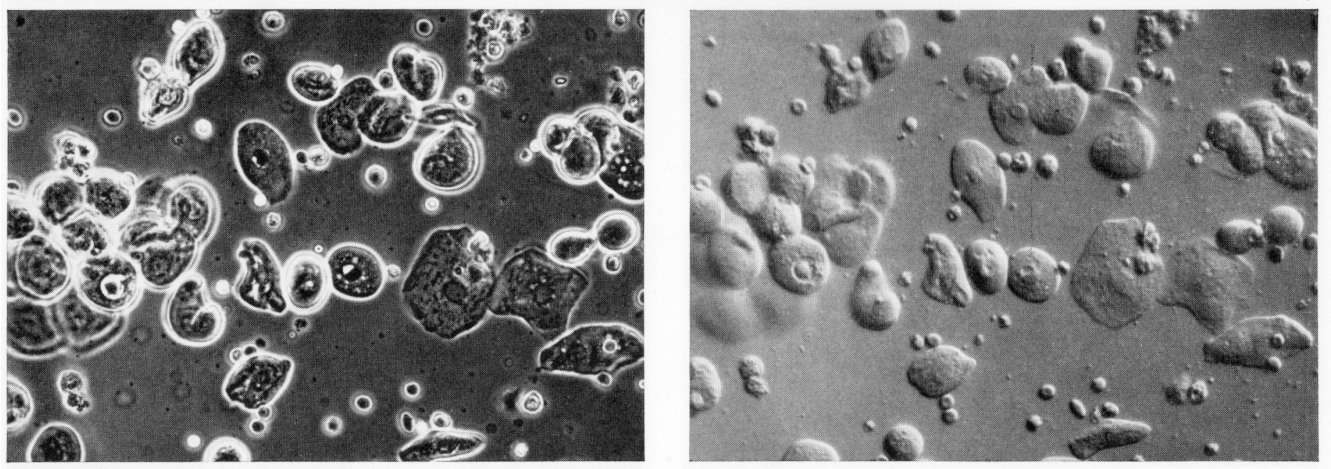
This is how the rear focal plane of the objective should look like when observed through an auxiliary microscope or the Photomicroscope's Ph Optovar setting if the DIC components are in correct arrangement. Turning the knob on the prism slider changes this colour through the interference spectrum.

The visible field of the specimen shows the same colours or shading across its entirety



If you see these beautiful colours, forget it: you have NOT true DIC**!**

(Photos F. Schulze)



Left: a phase contrast image and, right, the equivalent DIC impression.

Credits: all illustrations and tables not otherwise identified are courtesy of Carl Zeiss Oberkochen or Carl Zeiss Inc. New York.

I welcome any relevant questions but do not promise to have the answer!

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