



Use of the
C-Apochromat 40x/1,2 W Corr
with biological specimens
on the LSM 3/4

Operating manual

1. Goal

One of the main goals in confocal microscopy is to image 3D structures in biological specimens. Normally, the specimens feature a refractive index which is near to that of water ($n = 1.33$). The C-Apochromat has therefore been designed as a water immersion objective, allowing specimens of up to 220 μm thickness to be examined three-dimensionally.

The high demands made on the optical properties of this objective make it essential to be particular careful in application in order to enable full use of the performance of the objective.

In application, it is important

- to use cover slips of a known thickness;
- to precisely center the pinhole when using the objective with the LSM.

Cover slips No. 1 - H (ISO 8255/1), 0.17 mm, must be used. For exact measurements, the cover slip thickness must be determined first (see "Instructions for the determination of the cover slip thickness using the LSM"), and the correct thickness must then be set on the correction ring of the objective (see "How to proceed when using the C-Apochromat 40x/1.2 W Corr")

2. Instructions for the determination of the cover slip thickness using the LSM

In order to determine the cover slip thickness using the LSM 3/4, you should proceed as follows :

- Reflected-light reflection mode (no emission filters) and confocal mode: set $P = 10$.
- Set the correction ring on the objective to the thickness indicated by the manufacturer. If the thickness is not known, select the center position (160 μm).
- Set the contrast value to approx. 180. Search for reflection from the slip surface by focusing (first intensity peak when approaching the specimen). Optimize contrast.
- Select Z-line function. Set the parameters: "Number of Sections" = 500, "z-interval" = 0,4 μm , "Current Pos.Number" ≈ 460 . The "Refractive Correction" must be set to the ratio of the refractive indices of the cover slip and of water, i.e. $\text{RefrCorr} = 1.51/1.33 = 1.14$. The following illustration is the result from such a z-line measurement. The intensity peak of the underside of the cover slip (approx. 214 μm in the illustration) is slightly more pronounced than that of the surface (approx. 37.5 μm). The third and lowest peak (218 μm in the illustration) is from the surface of the object carrier. The "Measure" function in the "Ext. Rectangle" setting permits the distance between the two peaks, i.e. the cover slip thickness, to be measured directly as an y-value.
- Set the measured cover slip thickness on the Corr. ring of the objective. If the measured thickness differs considerably from the default thickness, repeat the measurement with the new setting.

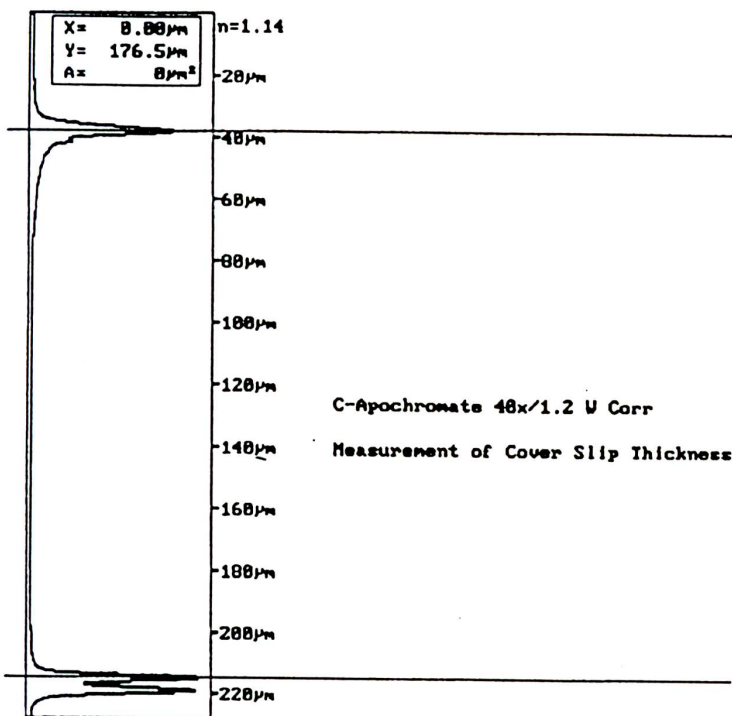
3. Procedure when working with the C-Apochromat 40x/1.2 W Corr

The correction range of the cover slip is (0.14...0.18) mm, with 2 μm increments and an excess range of (0.13...0.19) mm. The correction applies to temperatures $T_i = 23^\circ\text{C}$ (black marking) and $T_K = 37^\circ\text{C}$ (red marking).

The user exactly knows the thickness of the used cover slip (e.g. 0.160 mm) either from the manufacturer's indication or through his own measurement) and works at temperature $T_i = 23^\circ\text{C}$. He sets the black marking below the 0.16 mark, focuses and assesses his sample. Depending on the composition of the sample, the user moves the correction ring into the direction of thicker cover slips by small increments of 1-2 μm and observes the image during focusing. He changes the correction setting until the sample exhibits optimum contrast and resolution.

4. Cat.No. of the C-Apochromat

The catalogue number of the C-Apochromat 40x/1.2 W Corr is : 44 00 52.



z-line measurement for the exact determination of the cover slip thickness $y = 176.5 \mu\text{m}$

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