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MORE THAN MICROSCOPY

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SUPPORT

BASIC MICROSCOPY CONCEPTS

THE COVER SLIP

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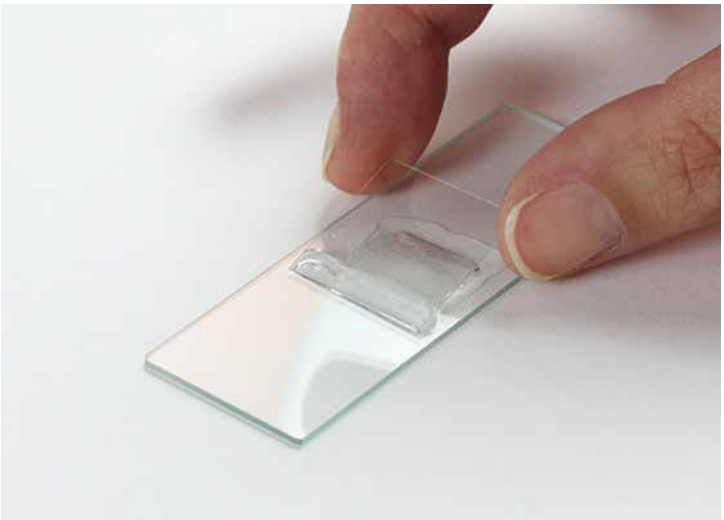
The uncommon yet simple rule “The sample defines the microscope” certainly helps to choose the appropriate microscope hardware for a defined application. Biomedical tissue sections or smears require a combination of upright microscope with transmitted light, while cell cultures (inverted plus transmitted light), flat and polished metal specimen (upright or inverted plus incident light), birefringent materials (upright POL plus transmitted light) or 3-dimensional biological beings (stereo microscope) request different microscope setups.

But there is a wider understanding of this guideline necessary to get the best possible image quality. A section of an organ tissue, a smear of a human mucous membrane, adherent or floating cells in microbiology: They all need a carrier or vessel to be evaluated under the microscope.

Sections or smears are fixed on a glass slide, in most cases conserved by a suitable embedding medium and fixed by a cover slip of defined thickness. Native biological samples (the leaf of a moss, pollen grains) can be placed in a drop of water with a cover glass on top. The proper microscope hardware in these cases is an upright model, while the sample additionally asks for a careful preparation.

Standard transmitted light objectives are designed for a 0.17mm cover slip between naked sample and front lens. This indication can be read on the objective sleeve **(1)**. Cover slips are industrial products with astonishing manufacturing tolerances (a stupendous fact in the world of optics where precise distances are essential!). The standard cover slips have a thickness of 0.13-0.16mm, taking the add-on layer of embedding medium or water into account to follow the 0.17mm rule **(2)**.

1 *Infinity optics, 0.17 cover glass correction*



2 *Placing a cover slip*

3

The higher the Numerical Aperture of an objective, the higher the sensibility for deviations from this value is. A 4X/0.10 or 10X/0.25 Plan Achromat may be used with or without cover glass without obvious visual effects. But taking a 40X/0.95 from Motic's new PLAN APO series **(3)**, we seriously have to take care for the best possible preparation.

Special cover slips with a tolerance of 0.17+/- 0.005mm are the best solution for that kind of demanding optics. No need to mention that a precise and thin sectioning (1-5 microns) remains a precondition. A voluminous portion of embedding medium destroys to potential improvement by these high quality cover slips. For smears of native samples in water a slight pressure of a dissecting needle helps to flatten the sample while unnecessary water may be sucked off by a paper towel.

You may like to avoid these efforts by using an immersion objective instead. Here the immersion medium homogenizes the space between naked sample and front lens, as its refractive index is similar to glass. The cooperation of embedding medium, cover slip and oil creates a homogeneous layer for best possible image quality. The disadvantages are clear: the need to avoid air bubbles (sometimes not easy as the front lens has a concave surface) and to clean the sample afterwards.

4

Time is money. Some users will choose a preparation method which does not require a final cover slip. Those samples can be treated by special objectives for "non-covered" specimen. This optics carries a "0" on the objective sleeve **(4)**.

5

In case of inverted microscopes, mostly petri dishes, flasks or well plates are sample carriers. The bottom of such plastic vessels has a thickness of 1mm. Consequently the required objectives are especially designed for inverted microscopes and carry the indication “1.1mm” **(5)** (The additional 0.1mm refers to the water/agar medium).

Due to the improved working distance, these objectives are not driven to maximum NA (resolution power). The inverted microscope with its restricted illumination aperture generally is not constructed for the evaluation of resolution limits, but for improved handling freedom. If you like to use a “0.17mm objective” on an inverted model (e.g. in case of fluorescence), take care to use a petri dish with 0.17mm glass bottom or turn the glass slide upside down. In case of an immersion objective gravity will not be on your side.

6

CONCLUSION

The objective designer has to know for what kind of optics he has to do his calculations: for glass slides with cover slip, for uncovered sections, aspirates and smears, for voluminous cell cultures. You can read helpful indications like “0.17”, “0” or “1.1” on the respective objectives. Our suggestion is to follow these “hints” for a satisfying image quality **(6)**.

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