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MORE THAN MICROSCOPY

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SUPPORT

BASIC MICROSCOPY CONCEPTS

RESOLUTION & MAGNIFICATION

1 20X / 0.40

2 20X / 0.50

3 20X / 0.65

RESOLUTION RULES MAGNIFICATION

Resolution may be defined as the ability of a reproducing system to separate individual signals, no matter what nature they are: e.g. optical or acoustical.

In widefield microscopy, resolution is understood as the ability of the microscope hardware, means optics, to separate individual events in a distance range down to roughly 220 microns. It is the objective which is the key element of any resolution calculation. Eyepieces and downstream digital cameras can only process the information flux which has entered the objective. The larger the opening angle of the objective, the more information is available for data processing.

The schoolbook tells us that the Numerical Aperture of an objective, indicated on the objective sleeve, is directly accessible for a calculation of the minimum distance which can be resolved. The following sequence of 20X lenses, starting from a Plan Achromat up to a Plan Apochromat, displays NAs from 0.40 **(1)** up to 0.65, **(2, 3)** thus increasing resolving power.

$$d_{min} \approx \frac{\lambda}{A_{objective} + A_{condenser}}$$

$$A_{objective} = A_{condenser}$$

$$d_{min} = \frac{\lambda}{2 \cdot A}$$

4

RESOLUTION RULES MAGNIFICATION

This formula has to be applied. (4)

d min is the minimum distance which can be resolved, NA the numerical aperture of objective and condenser respectively. In an ideal case both angles are identical (NA is the sinus value of a half opening angle of the objective). So taking into consideration that the human eye is most sensible in the range of 550nm (green), for a 20X objective we may calculate as follows:

Plan Achromat 20X
d min = 550nm/2 x 0.40 = 688nm

Plan Fluorite 20X
d min = 550nm/2 x 0.50 = 550nm

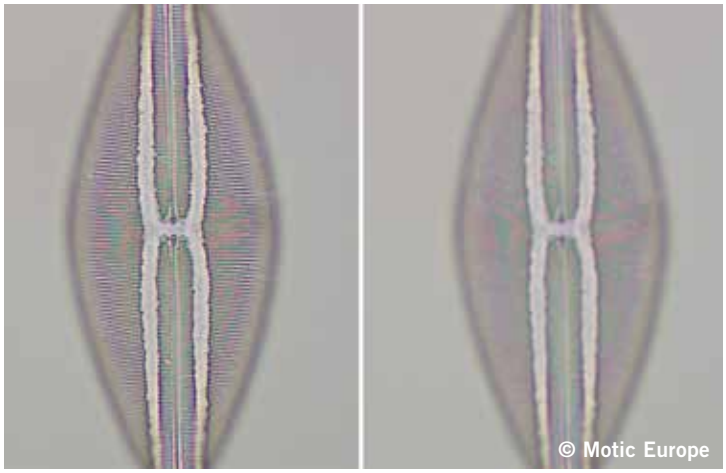
Plan Apochromat 20X
d min = 550nm/2x 0.65 = 423nm

So the resolution power for a 20X objective will be maximum 423nm. Taking into consideration that the aperture diaphragm of a condenser will have to be closed for contrast reasons, this ideal calculation will not be verified. But it gives some idea about the limits of conventional light microscopy.

It may be worth to mention that the user can do a lot to maximize the image results. Any deviation from the standard cover slip thickness of 0.17mm, any overload of embedding media of the sample (which acts an additional cover slip), any grease or oil on the front lens of a dry objective will have a deep impact on the image result.

These images **(5)** display a diatom, a monocellular alga, enclosed in a silica exoskeleton with tiny pores of specific size. The left image resolves perfectly the arrangement of the pores, while the right image with the same magnification power is not able to display details.

Resolution, not magnification is the key issue.



5 Diatom enclosed in a silica exoskeleton with tiny pores

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