

Diffraction-limited Resolving Power

When the resolving power of an imaging system is only limited by the equation given by Ernest Abbe (1840 -1905), its resolution is diffraction-limited. The Abbe limit (d) is defined by wave length (λ) and the numerical aperture (N.A.) of objective and condenser lenses.

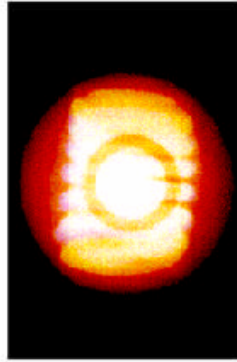
$$d = 1.22 \times \lambda / (\text{N.A.}_{\text{obj}} + \text{N.A.}_{\text{cond}})$$

In microscope, illumination light wave suffers from diffraction at the object plane of object plane. The panel below illustrates the diffraction pattern formed at the objective lens' intermediate image plane.

Light Rays and Diffraction



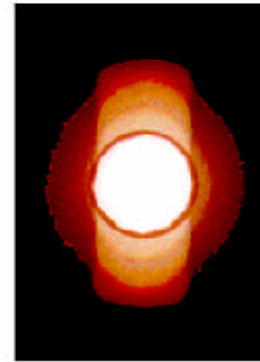
Objective micrometer placed on sample stage
Nikon Optiphot
Plan 40x/0.55 objective,
N.A. 1.25 Phase
condenser



Filament image formed at intermediate plane of the objective



Condenser's annular ring image formed at the intermediate image plane of objective



Diffraction pattern generated by the objective micrometer formed at the objective's intermediate image plane

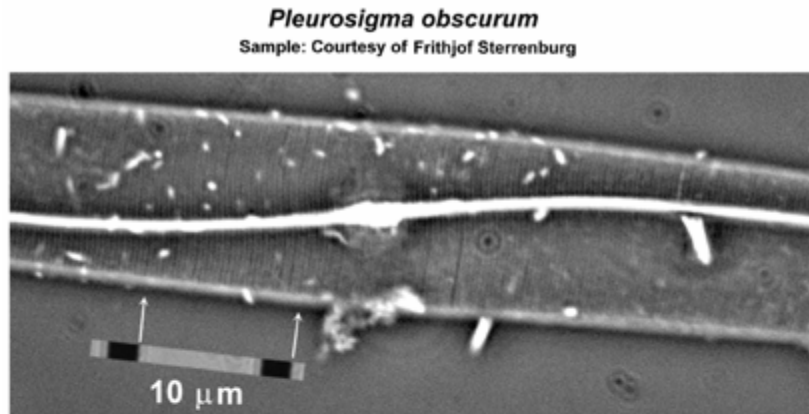
(Y.F. Feb. 2002)

Note that theoretical value of spatial resolution only indicates the maximum possible resolution. There are many factors affecting the actual resolving power, including filters (wavelength), condenser, Köhler illumination, quality of objective lens, type of immersion oil, and the refractive index of the specimen. Seeing a fine detail with our eyes not always guarantee that the detail is precisely transferred into recording medium such as film or imaging sensor. Below is an example how the resolving power was proved being the maximum achievable level using a 100x, N.A. 1.3 objective in conjunction with N.A. 1.3 condenser when the sample was properly illuminated with 546 monochromatic light. It is also

necessary that the resolution of image file is properly adjusted to match the monitor resolution when viewing the result on computer monitor.

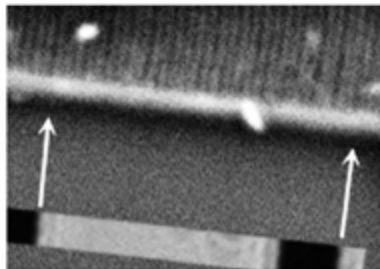
For more detail, see links: "[Aliasing.pdf](#)", "[Moire Pattern.pdf](#)", "[Matching resolution.pdf](#)", "[ModulationTransferFunction.pdf](#)", and "[Human eye.pdf](#)" in "Technical Notes" Section (08-TechNote.html).

Theoretical maximum resolution: $d = 1.22 \times 546 / (1.3 + 1.3) = 256 \text{ nm}$
Width of dot line of *Pleurosigma obscurum* silicate: 250 nm

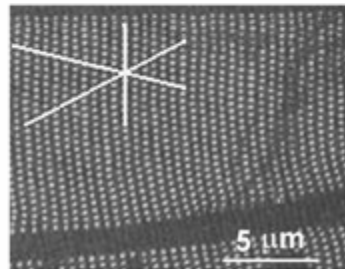


Axioskop-50/Photometrics PXL (1317 x 1035 pixels);
Neofluar 100x/N.A. 1.3 Ph3 objective,
N.A. 1.4 (dry) Achromatic-aplanatic condenser.

Dot line width: 250 nm



High magnification showing
the resolving power of 250 nm



SEM micrograph showing
exact silicate arrays
(Courtesy: F.A.S. Sterrenburg)

The author is most thankful to my honorable friend, Dr. Frithjof Sterrenburg of Netherlands, for providing the diatom and SEM micrograph used in this page.

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