

The paper presents the mathematical technique for calculation of the diffraction depth of focus of an optical system of a widefield microscope. The proposed technique applies the Rayleigh criterion based on evaluation of the wave aberration appeared due to defocus in a high aperture optical system. The maximal value of a linear approximation of the defocus wave aberration is used to define the depth of focus. It is proven that in optical systems with numerical aperture higher than 0.5 have the diffraction depth of focus 25 - 40 % smaller than the widely known formula defines. This fact is important for implementation of autofocus and digital focus extension algorithms. The nonsophisticated formula for calculation of the depth of focus is proposed. The results of experimental measurements of the depth of focus are presented and discussed.

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