Microscope point spread function, focus and calculation of optimal microscope setup

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In microscopy exists special distance between sample and objective, which we called focus. Using the diffraction integral analysis we may define the position of maximal intensity (theoretical focus) as well as shape of the energy intensity profile along the lense axis. This is called

During our experiments we defined three different types of focus. The first one is the position of maximal light intensity. Apart from the light intensity this position is also characterized by projection of the image object in the shape most similar to Airy rings: the *Airy focus*. By focusing just by human eyes we, however, seldom place the working position of the object into this theoretical position. This "expert defined" focus we call *human eyes focus*. Using our image entropy approach (Stys et. al. 2011) we were able to calculate objective value for the user defined focus as the point in which the image carries most information.

By visual as well as objective (image entropy calculation) examination it is possible to reconstruct the arisal of this position: it comes from the fact that observed objects have finite thickness. The points below and above the focal plane are projected as alternate white and dark spots, however, in one direction respective to the focus they become more distorted. The tendency of the operator is to position the focus in the region where the images are distributed equally below and above the putative inflection point at which the diffraction image is neither dark nor white. This position is not unique due to various lense aberrations and specific projections of different colours. Thus, we consider the "*information focus*" a best compromise for each given sample. Moreover, in case that a standard object is placed in the image, calculation of information focus may be used for universal objective setup and comparison between images.

In the paper we demonstrate arisal of the information focus using standard microscopic objects – latex particles. We also give several examples of the use of the information focus for setup of the microscope for capture of living cell images. The calculations are performed by software tools developed on our institute. In the paper will be also given description of their features related to microscopy. The method is, however, very general and may be used for any type of imaging and image capture as will be outlined.

Stys D., Urban J., Vanek J., Císar P. Analysis of biological time-lapse microscopic experiment from the point of view of the information theory. Micron. 2011a, 42, 360-365