All you've ever wanted to know about spatial resolution and how to measure it

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Abstract

Spatial resolution is usually the main feature that is sought by fluorescence microscope users. Historically, due to the commercial availability of fluorescent beads, the resolution limit of fluorescence microscopes has been associated to the measurement of the full-width at half maximum (FWHM) of the point spread function (PSF). This approach is known as the single-point resolution approach. However, there are other approaches to measure spatial resolution: the two-point, the single-line, the two-lines, and the optical transfer function approaches can also be used, each one having its own advantages and drawbacks.

In this workshop, we will come back to the original definition of resolution. We will then highlight the experimental parameters that influence this feature, such as background and noise. We will present different approaches to measure it – the single-point, the two-point, and the contrast transfer function approaches - and their associated available tools – fluorescent beads, DNA nanorulers, and gradually spaced lines from Argolight products.

Special attention will be brought to images of gradually spaced lines that have been either acquired by structured illumination microscopy or processed with algorithms (such as deconvolution) to improve resolution.

The workshop will be a mix of theoretical considerations, presentation of experimental results, and demonstrations using Argolight image analysis software, Daybook.