NEW APPROACH TO MEASURE THE RESOLUTION LIMIT IN CONFOCAL FLUORESCENCE MICROSCOPY

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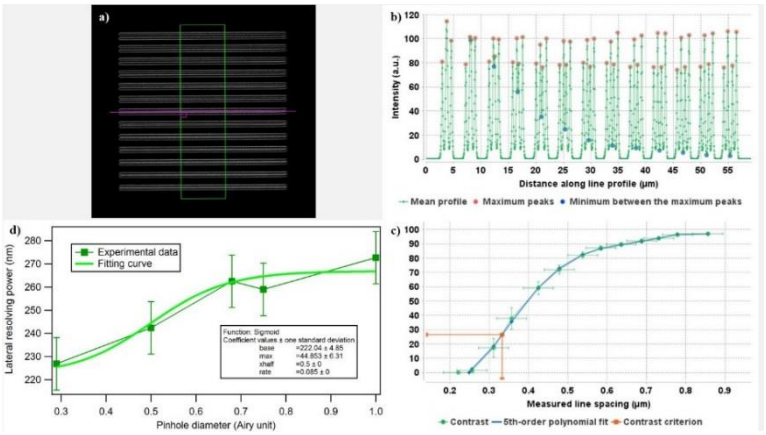
Historically, due to the commercial availability of fluorescence microspheres, the resolution limit of a fluorescence microscope has been associated to the measurement of the full-width at half maximum (FWHM) of the point spread function (PSF) at the center of the field of view. This approach is known as the single-point resolution approach. The theoretical description of this approach in the confocal fluorescence microscope is well known [1], and a very detailed protocol to measure and interpret the PSF is available [2]. However, there are other approaches to measure the spatial resolution [3]: 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, drawbacks, and associated reference samples.



In this work, we present a new way for measuring the spatial resolution of confocal fluorescence microscopes, based on the two-line approach [4]. The reference sample associated to this approach is an Argolight Argo-HM quality control slide, containing long-term fluorescent multicolor patterns at the sub-µm scale, both in 2D and 3D, embedded in glass [5]. In particular, gradually spaced lines are present, consisting of lines separated from 100 to 700 nm, by step of 50 nm. A fully automated algorithm, associated to this pattern, and freely useable in the Argolight software Daybook-Analysis, has been developed to measure the lateral resolution. From a confocal fluorescence image of the gradually spaced lines (a), the algorithm extracts an intensity line profile (b), perpendicular to the lines, detects the peaks (in red) and the valleys (in blue), measure the contrast, and plot it in the contrast transfer function (c), *i.e.* the contrast values versus the line spacings. Due to the stability of the pattern, studying the evolution of the spatial resolution versus the pinhole size (d) and of the evolution of the resolution over time is possible.

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