IMPROVING THE LATERAL RESOLUTION IN FLUORESCENCE CONFOCAL MICROSCOPY USING LATERALLY INTERFERING BEAMS

O. Haeberlé and B. Simon
Laboratoire MIPS - Groupe Lab.El, Université de Haute-Alsace
IUT Mulhouse, 61 rue Albert Camus, 68093 Mulhouse Cédex, France
E-mail: o.haeberle@uha.fr

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The resolution in optical microscopy is limited by the diffraction phenomenon. Ernst Abbe [1] introduced in the late 19^{th} century his resolution criteria (for classical microscopy) as:

$$R_{lat} = 0.5\lambda/NA$$

λ being the observation wavelength, and NA the numerical aperture of the objective. For years, this limit was considered as ultimate. Marvin Minsky [2] made a first breakthrough by inventing the confocal microscope. Recently, new techniques like structured illumination [3] and STED microscopy [4] have permitted to further improve the resolution in 3-D fluorescence microscopy. However, these very promising techniques up to now still have some limitations. Structured illumination requires taking several images of the same specimen to recompose a resolution-improved image, which may be a drawback for bleaching-sensitive dyes, while STED microscopy, has been proven for a small number of dies only.

We propose a simple technique to improve the lateral resolution for 2-D or pseudo 2-D specimens, like for examples microtubules after extraction from a cell, or chromosomes in the metaphase state, deposited on a glass slide. With properly prepared samples, and using the objectives with the highest numerical aperture available combined with a simple halves phase plate [5] to modify the excitation beam in a confocal microscope, a lateral resolution of 90 nm is possible for existing fluorophores. Applied to new dies, sensitive to 2-color 2-photon excitation [6], the resolution may be improved to 60 nm.

For 3-D observation of living specimens, the use of water immersion objectives, with lower NA and therefore lower resolution is mandatory. The 4Pi microscope [7] as permitted to dramatically improve the longitudinal resolution to about 80 nm in some cases. However, the lateral resolution is still the same as a regular confocal microscope (≈140 nm). We show how using laterally interfering, x-polarized excitation beams permits in theory to obtain a (y-z) 2-D resolution better than 100 nm. In a second step, using y-polarized excitation beams permits to obtain an improved (x-z) 2-D resolution. Then, image recombination (data fusion) permits to obtain an isotropic 3-D resolution slightly better than 100 nm. The main advantage of this approach is that it consists in an evolution of the established 4PiC technique [8].

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