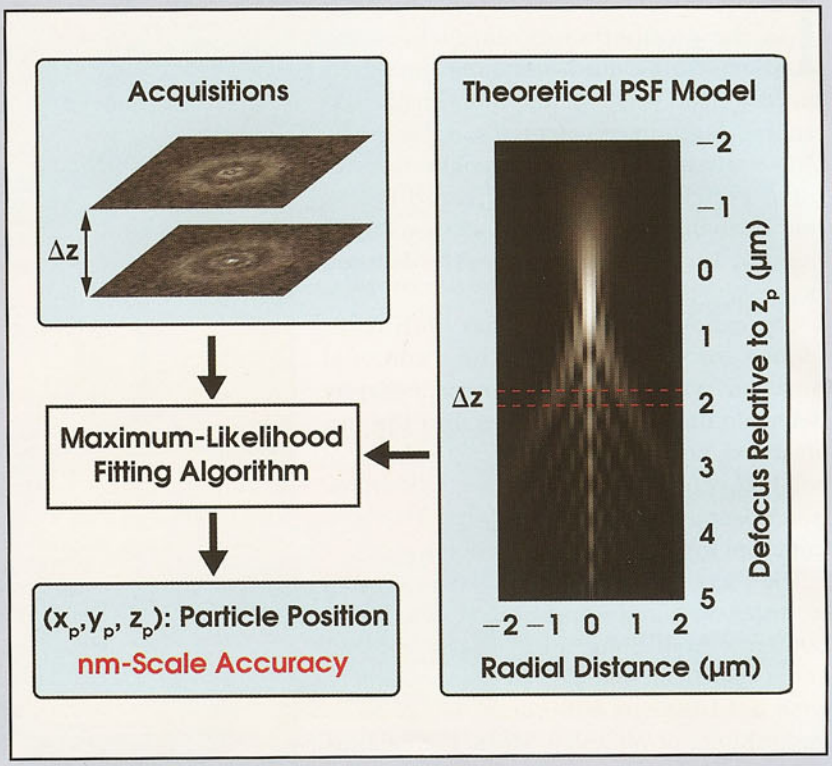
Accurate Localization in Fluorescence Microscopy

At Ecole Polytechnique Fédérale de Lausanne in Switzerland, scientists have developed a technique for accurately tracking fluorophores in wide-field fluorescence microscopy that requires no customized hardware. It localizes particles to a precision better than 15 nm in the axial direction, and has applications in studies of molecular dynamics and interactions of living cells.

The approach uses the diffraction rings in a stack of defocused images taken at various focal distances, and it takes into account the aberration in the microscope and the noise in the imaging camera. The model-based technique compares the actual diffraction pattern with that predicted for a particle at a given position, and iterates to find the position of maximum likelihood.

The published results present both theoretical and experimental evidence of the resolving capability of this method. The team used a Zeiss Axioplan 2 microscope with plan-apochromat oil immersion objective for 63× magnification and an Axiocam CCD camera to record the images. Molecular Probes TetraSpeck fluorescent microspheres provided the imaging targets, and a Leica TCS SP2 AOBS confocal microscope confirmed the calculations of their axial positions. Matlab software from The Mathworks implemented the algorithms.



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