Axial Tomography in Live Cell Microscopy

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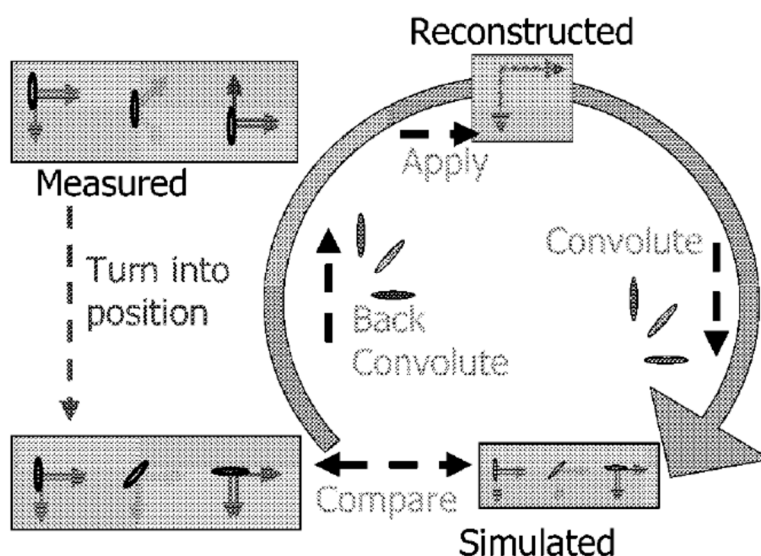[4]   Institute of Molecular Biology (IMB), 55128 Mainz, Germany
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Computational Reconstruction of 3D images from axial tomography data

A mathematical analysis of the likelihood for an axial tomographic measured data set resulted in the following iterative reconstruction process: In a first step, all data sets have to be rotated and translated in a way that their geometry is aligned; the PSF used for data reconstruction has also to be turned by the same angles. The center of intensity of the PSF, however, is not changed. Then an initial "guess" data set (G0) is chosen. It proved to be useful to choose the data set to be equal to a constant (one). Another possibility would be to choose the mean value of all data sets. One iteration step number n consisting of a forward projection P of the guessed data set is calculated. This is done by computation of a convolution of the actual guess G_n with the microscopical point spread function PSF. This convolution is computed with PSFs, which were adapted to the rotation angle for every measured data set imaging orientation . Then the correction value of $C_i = M_i / P_i - 1$ is calculated for each pixel i in every view, where M_i is the measured value. This correction value is then projected back by a convolution 2 with the room-inverted point spread function $PSF(-\vec{x})$. The back-projected correction C_i is then applied to the actual guess using

$$G_i^{n+1} = G_i^n + q\tilde{C}_i G_i^n.$$

The over-relaxation factor q is used for improving the speed of convergence of the algorithm. This iteration process is depicted in the Supplementary Figure S1.

Supplementary Figure S1. Flowchart of the axial tomographic maximum likelihood algorithm. Convolutions are always performed using rotated PSFs. From R. Heintzmann R.; Kreth, G.; Cremer, C. Reconstruction of axial tomographic high resolution data from confocal fluorescence microscopy: a method for improving 3D FISH images. *Analytical Cellular Pathology* **2000**, 20, 7–15. ISSN 0921-8912.