Purpose:
Accurate study of 3D cell morphology with confocal imaging method requires correction of the z-axis aberration. A fast method with fluorospheres has been developed to achieve this goal.
Methods:
Fluorospheres with nominal diameter of 10um(+-2%) were used for this study. The z-stack images were obtained using Zeiss-410 CLSM system with the stepsize of 0.4 um in air along the z-axis. Ideally, a sphere should be shown as a disk with diameter d in each image of the stack. But the measured data showed significant deviation in d vs z relation from the expected one due to the optical aberration by light refraction at various index-mismatched interfaces. To correct this aberration, the following algorithm were developed and used to fit the measured data and obtain a rescaling factor for accurate 3D reconstruction along z-axis.
We define three parameters, 1. Covariance between theoretical and experimental data of the disk diameter arrays (Pcov), the maximum of which means the optimal match with the theoretical model; 2. Eccentricity of the largest disk which is in the middle layers and less influenced by the PSF (Pecc); 3. The mean eccentricity of all the stacks measured (Pmecc). These parameters were utilized and weighted with factors of a, b and c, respectively, in the objective function (F).
$F = a*Pcov + b*Pecc + c*Pmecc$
Results:
29 images were acquired for the fluorosphere as the measured image stack. After the data fitting and optimization, diameters in all images were corrected to obtain the rescaling factor (f=0.862), and the aberration was reduced significantly in the reconstructed 3D image of the fluorosphere.
Conclusions:
It is shown that the above method can significantly improve the quality of the reconstructed 3D image of the fluorosphere from the confocal images. Additional test results will be presented for evaluation of the method's effectiveness.