Lab 9: 3D deconvolution of the microscopy fluorescence image

INTRODUCTION:

In modern cell biology community, most three-dimensional (3D) fluorescence microscopy images are now obtained by the confocal microscope. Confocal microscopes are better than conventional fluorescence microscopes because the confocal design reduces haze from fluorescent objects not in the focal plane. Although confocal microscopy has many advantages, it does have limitations. A serious drawback for some applications is the amount of excitation light required to produce a confocal image. This may be a problem for fixed specimens that require many focal plane images or for fixed specimens that are labeled with several different dyes. In these cases, the excitation-light dosage required to obtain satisfactory 3D images may bleach the dye.

Deconvolution is another way to eliminate the fluorescent light out of the focal plane. A large portion of early deconvolution algorithms were based on a known imaging system response, namely the point spread function (PSF), which can be measured or estimated. The most classical method in such area was the linear inverse-filtering algorithm which states that the deconvolution operation of the PSF from the blurred image in the space domain is equivalent to the division in the Fourier domain; other linear methods such as Wiener filtering, linear least squares algorithm and Tikhonov filtering were also available. Nonlinear iterative methods such as Janson Van Cittert algorithm, nonlinear least-squares algorithm and constrained Tikhonov-Miller algorithm were proposed to improve the deconvolution performance.

In this lab, you will learn to implement the inverse filtering, Wiener filtering and Maximize the likelihood (R-L iteration) on the simulation data and real data.

OBJECTIVES:

1. To learn the implementation of inverse filtering and Wiener filtering.

2. To compare and understand the different results of different methods.
3. To learn the implementation of the R-L iteration. (Optional)

MATERIALS:

Fiver '.mat' files are provided in this case, including, a non-blurred microsphere, a calculated PSF data, a calculated blurred microsphere without noise, a measured PSF data and a measured microsphere data.

TASKS:

1. Generate noise disturbed data using calculated blurred microsphere data; the MATLAB function 'imnoise' can be used here, and the 'gaussian' and 'poisson' noise models are recommended here; Be careful,
    1) the original calculated blurred microsphere data assumes that the fluorescence intensity of each point is 1 and this fluorescence intensity should be adjusted to ensure the final value of image pixels are not exceed 1;
    2) it is recommended to add background noise to simulate the real detector system.
2. Implement the inverse filtering and Wiener filtering method, the MATLAB function 'deconvwnr' is not allowed.
3. Implement the R-L iteration algorithm. (Optional)
4. Compare the inverse filtering, Wiener filtering and R-L iteration on simulated data; the R-L iteration method can applied based on either of TASK 3 and MATLAB function 'deconvlucy'; try to change the noise model and NSR to compare and discuss the different results.
5. Apply the inverse filtering, Wiener filtering and R-L iteration on real data and compare the results.

LAB REPORT:

1. The lab report should include but is not limited to:
    1) Introduction and objectives.

2) Principles.

3) For every task:

   i. Describe the important details of your codes.

   ii. List the core codes and describe the functions.

   iii. Results and discussion.