BME42-731/ ECE18-795/CB02-740 Bioimage Informatics
Spring 2014
Project Assignment #2

Pixel-level and subpixel-level particle feature detection

Assigned on Feb-17-2014

➔ ***The report is due on Mar-10-2014 in class or by 5PM at TA's office.***

➔ ***This project is to be completed by groups.***

➔ ***Groups 1 & 3 are required to give presentations on Mar-12-2014 in class.***

A. Overview

The main goal of this project is to implement pixel-resolution and subpixel-resolution particle feature detection. It is divided into two parts. In the first part, you are asked to implement the pixel-resolution particle detection algorithm as described in reference [1] and discussed in class. In the second part, you are asked to implement the Gaussian kernel fitting algorithm for sub-pixel resolution particle detection, as described in reference [2] and discussed in class. The total score for this project is 80 points. There is also an extra credit question for 15 points.

B. Instructions

B.1 Image data

The image sequence can be downloaded from the Blackboard portal for this class (under "Assignments". For initial implementation and testing, use one frame from the sequence. However, you final implementation should detect particles from all frames.

B. 2. Part I: Particle feature detection

B.2.1 Calibration of dark noise (10 points)
- Manually crop a rectangular region in the image background area, which we assume contains background noise. Calculate the mean and standard deviation of background noise. These parameters will be used next.

B.2.2 Detection of local maxima and local minima (10 points)

- Filter each frame with a Gaussian kernel with standard deviation equal **one third of** the Rayleigh radius. The image sequence was collected using an objective lens with 100× and a NA of 1.4. The fluorophore used is YFP (Yellow Fluorescent Protein). Assume its excitation wavelength at 527 nm. **Assume a pixel size of 65nm.**

- Use a 3×3 mask to detect local maxima and local minima.

- Select one frame; compare detection results using a 3×3 mask versus a 5×5 mask.

B.2.3 Establishing the local association of maxima and minima (10 points)

You can use either a Delaunay triangulation or a nearest neighbor approach. If you use a nearest neighbor approach, select the nearest 3-4 local minima.

B.2.4 Statistical selection of local maxima (15 points)

Implement the t-test based statistical selection of local maxima as in [1]. The confidence quantile should be a parameter that users can select. **For simplification of implementation, crop a background region and calculate the background noise (see B.2.1). Consider only this noise in your t-test (i.e. ignore shot noise, as it is low). Specifically, set the term of $\sigma(I_L)$ in Equation 4 of reference [1] to zero. Use the noise parameter of the cropped background region as $\sigma(I_{BG})$.** Further implementation details will be discussed in class.

Run your detection program to process the image sequence provided. Save the result for each frame into a separate .mat file.

B.3 Part II: Sub-pixel resolution particle detection

The implementation essentially follows the scheme described in reference [2].

B.3.1 Choose detected particle positions and intensities from Section B.2.2. Generate a synthetic image using these positions and intensities as ground truth (15 points)

Generate a raw image by using the coordinates of detected particles in the first frame of the image sequence used in Section B.2.2. Convolve the raw image with a Gaussian that approximates the PSF to generate a synthetic image. **Assume the same imaging parameters as in B.2.2 when calculating size of the Gaussian.** Simulate actual image noise by adding white background noise at a level you define. Further implementation details will be discussed in class.

B.3.2 Implement a sub-pixel resolution detection algorithm using pixel oversampling (20 points)

Perform a subpixel resolution particle detection on the image sequence provided. You are asked to implement the Gaussian kernel fitting algorithm in [2]. **For implementation, oversample the pixel size to 13nm (i.e. oversample the original image by a factor of 5).**

B.3.3 Benchmarking subpixel resolution particle detection

Apply your subpixel particle detection implementation to the synthetic image ground truth generated in B.3.1. Quantify detection accuracy and precision by calculating the mean and standard deviation of detection error. (Extra credit 15 points)

C. Instructions on preparing your report

1) Organize your report following the sequence of questions. Whenever possible, include representative results for each question in the report, and briefly explain and/or comment on your results.

2) Submitted MATLAB code should be clearly formatted and commented for ease of reading and grading.

3) Submit all relevant images and/or videos generated for each assignment.

D. Report format

There is no page limit for the project assignment report. Please follow the recommended report format, as described in Lecture 09.

Page size: letter
Line space: single
Page margins: 1 inch on each side (top, bottom, left, right)
Font size: 12 points font for the main text; 10 points for listed references

E. Submission of MATLAB code

Our TA has set up upload links for result submission. Please let us know if you encounter any difficulties.

Reference

[1] A. Ponti, P. Vallotton, W. C. Salmon, C. M. Waterman-Storer, and G. Danuser, Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy, *Biophysical Journal*, 84:3336-3352, 2003.

[2] M. K. Cheezum, W. F. Walker, and W. H Guilford, Quantitative comparison of algorithms for tracking single fluororescent particles, *Biophysical Journal*, 81:2378-2388, 2001.