Biological Image Processing and Informatics

Spring 2023
Project Assignment #2

Assigned on April-13-2023. Due May-07-2023 10:00PM

➔ ***This project is to be completed by groups. Each group can have up to three members.***

A. Overview

This project consists of three parts. Part I introduces implementation of different forms of Gaussian filters for image noise suppression and image derivative calculation. Part II introduces particle detect at pixel resolution and sub-pixel resolution.

The total score for this assignment is 130 points. You will also get 20 extra credit points if you successfully complete question 3.5.

NOTE: **This assignment is for groups NOT individuals. Each group only needs to submit one report.**

Instructions

PART I: Spatial filtering and derivative calculation using Gaussian kernels

1.1 (20 points): Implementation of a Gaussian filter

Implement a Gaussian filter with its standard deviation σ as a user input. Remember to normalize the filter. Apply Gaussian filter with σ=1, 2, 5, 7 to the image "axon01.tif" of Project Assignment 01. Compare the result images at these variance levels.

1.2 (20 points): Calculate image intensity derivatives

Use the image "axon02.tif" and "cell_nucleus.tif" from Project Assignment 01. Following what is covered in the class, calculate the derivative in the horizontal and vertical direction using convolution with the first order derivative of a Gaussian kernel under σ=1, 2, 5. Display the derivative images.

PART II: Generation and fitting of Airy disks

2.1 Implement a MATLAB function to plot the Airy disk.

The function should take the numerical aperture and light wavelength as two input variables. For simplification, normalize its peak magnitude to 1. Plot and compare the Airy disks for the following configurations (please plot each using a different color for comparison). What conclusions can you draw from comparing the Airy disks (20 points)

1) λ= 480, NA = 0.5
2) λ= 520, NA = 0.5
3) λ= 680, NA = 0.5
4) λ= 520, NA = 1.0
5) λ= 520, NA = 1.4

6) λ= 680, NA = 1.5

2.2 Fitting of the Airy disk using a Gaussian kernel
Based on your implementation in 3.1, expand to include an option to find the best fit of the plotted Airy disk using a Gaussian kernel. In particular, for each of the six listed configurations, find the standard deviation σ of the Gaussian kernel that gives the best fit, and compare σ to the radius of the Airy disk. What conclusion can you draw from this comparison? (20 points)

PART III: Particle detection at pixel resolution and sub-pixel resolution

Use the image sequence provided. The sequence contains 5 frames.

Pixel resolution particle detection

3.1 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.

3.2 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 515 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.

3.3 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.

3.4 Statistical selection of local maxima (20 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. 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.

Sub-pixel resolution particle detection (EXTRA CREDIT 20 points)

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

3.5 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. Instructions on preparing your report

1) Write a project report following the format listed below. Upload the report in PDF to Canvas.

   The report should include the following sections:
   - *Project number and title, student names, date of submission*
   - *Introduction: write a general and brief summary of the project.*
   - *Code execution instruction: provide clear instructions on how to run your code.*
   - *Result section: present all the key results. Present these results by showing the images. This part should be organized largely following the sequence of questions. Concisely explain/comment on your results.*
   - *Summary/discussion section: summarize and discuss what you have learned from this project.*
   - *References: list all the references you want to cite.*

2) Organize your report according to the sequence of questions. Whenever possible, show representative results for each question into the report, and briefly explain and/or comment on your results.

3) As a requirement for good practice in programming, your MATLAB code should be properly formatted and commented.

4) Submit all relevant images and videos generated for this assignment to Education Cloud.

C. Report format

There is no page limit for the project assignment report.
Page size: letter
Line space: single
Page margins: 0.5 inch on each side (top, bottom, left, right)
Font size: 11 or 12 points font for the main text; 10 points for listed references

References

[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 flurorescent particles, *Biophysical Journal*, 81:2378-2388, 2001.

[3] I. T. Young, Quantitative microscopy, *IEEE Engineering in Medicine and Biology*, pp. 59-66,1996.