BME42-731/ ECE18-795/CB02-740 Bioimage Informatics
Spring 2015
Project Assignment #2

Assigned on Feb-11-2015

- ➔ ***The report is due on March-09-2015 in class or by 5PM at TA's office.***
- ➔ ***This project is to be completed by assigned groups.***
- ➔ ***Two groups will be selected to give presentations on March-11-2015 in class.***

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 operations for characterizing basic properties of microscope systems, such as background noise, illumination uniformity, and pixel-size. Part III reviews basic concepts of point spread functions and Airy disks

The total score for this assignment is 160 points. You will also get 20 extra credit points if you successfully complete question 1.3.

NOTE: This assignment is for groups NOT individuals. Each group only needs to submit one report. Two groups will be selected to present their results.

B. 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 variance σ as a user input. Remember to normalize the filter. Apply Gaussian filter with σ=1, 2, 5, 7 to the image in Question 2 of Project Assignment 01. Compare the result images at these variance levels.

1.2 (20 points): Calculate image intensity derivatives

Use the image from Question 2 of 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.

1.3 (extra credit 20 points): Implementation of a directional anisotropic filter

Implement the anisotropic Gaussian filter described in the following paper (only the non-iterative form).

J.-M. Geusebroek, A. W. M. Smeulders, and J. van de Weijer, Fast anisotropic Gaussian filtering, *IEEE Trans. Image Processing*, vol. 12, no. 8, pp. 938-943, 2003.

Use the image from Question 2 of Project Assignment 01. Apply an anisotropic filter at 30, 60, 90, 120, 150 degrees with σ=10 in the longitudinal direction and σ=5 in the lateral direction. Display the result images.

PART II: Basic operations for microscope characterization

2.1 Downloading image data

Images for this project can be downloaded from Blackboard. The files (as a compressed file) can be found next to the instruction file.

2.2 Characterizing fluorescence image background noise (30 points)

- Write a program that first crops a rectangular region from the background of the image series and saved the cropped region into a series of images that are named sequentially (such as background001.tif, background002.tif, …). Remember to turn off compression when saving the images. (5 points).

- Analyze the cropped background noise data to address the following three questions (20 points)

  2.2.1 What distribution does the noise signal follow? Is it a normal distribution? Is the noise white? Why? (10 points)
  2.2.2 Does the noise distribution change over time? (5 points)
  2.2.3 Does the noise distribution change over space? (5 points)

- Answer question 2.2.1 on background noise of the two images from JCB viewer that are used in Project Assignment 01 (5 points).

2.3 Characterizing illumination uniformity (20 points)

Define and implement you own quantitative descriptors to characterize the uniformity of illumination. (Hint: analyze image intensity distribution in the background area with no image features)

2.4 Microscope pixel calibration (30 points)

As demonstrated in the lab visit, a micrometer slide from TedPella is used for calibration. Detailed specifications can be found at http://www.tedpella.com/histo_html/2280-10.htm The slide that we use is No. 2280-16.

  2.4.1 Develop a manual/interactive approach to calibrate pixel size (20 points) under different magnifications.

  2.4.2 Develop a semi or fully automated method to calibrate pixel size (10 points)

PART III: Generation and fitting of Airy disks

3.1 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

3.2 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)

C. Instructions on preparing your report

1) Write and submit a report in printed copy. There is no required format. But the report should generally follow the sequence of questions.

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. We will use the same BOX folder for result uploading.

D. Report format

There is no page limit for the project assignment report.
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