

## **BME 42-731 / ECE 18-795 Project Assignment #2**

### **Statistical and sub-pixel particle feature detection**

Assigned on Feb-15-2010,  
*Due on Mar-15-2010 in class*

#### **A. Overview**

This project is divided into two parts. In the first part, you are asked to implement the statistical particle feature detection algorithm described in Lecture 8. In the second part, you are asked to implement one of the sub-pixel feature detection algorithms described in [1]. The total score is 80 points.

#### **B. Instructions**

##### **B.1 Image data**

We will use the same *Drosophila* vesicle transport image sequence that we used in project assignment 1.

For the initial implementation and testing, you only need to use one frame from the sequence. However, your final implementation must be able to detect particles from all 150 frames in a batch.

##### **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 essentially noise. Calculate the mean and standard deviation of background noise. These parameters will be used later in the statistical selection of point features in B.2.5.

###### **B.2.2 Low pass filtering using a Gaussian kernel (10 points)**

- Implement a 2D Gaussian filter. Be sure to normalize the coefficients so that they add up to 1.

- Apply the Gaussian filter with standard deviation of 1, 2, and 5 to one selected frame from the image sequence; Identify cases when point features in unfiltered image become merged or shifted.

###### **B.2.3 Detection of local maxima and local minima (10 points)**

- Apply a Gaussian kernel with standard deviation equal the Rayleigh radius. The image sequence was collected using an objective lens with 100 $\times$  and a NA of 1.4. The

fluorophore used is YFP (Yellow Fluorescent Protein). Its emission wavelength is at 530 nm.

- Use a  $3 \times 3$  mask to detect local maxima and local minima.
- Select one frame, compare detection results using a  $3 \times 3$  mask versus a  $5 \times 5$  mask.

#### **B.2.4 Constructing 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.2.5 Statistical selection of local maxima (15 points)**

Implement the t-test based statistical selection of local maxima as in [2]. The confidence quantile should be a parameter that can be set by users. Further implementation details will be discussed in class.

Run your detection program over the 150 frames. Save the result for each frame into a separate .mat file. We will use this result for project assignment 3 in which we will implement two tracking algorithms.

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

**The implementation follows the scheme described in [1].**

#### **B.3.1 Based on detected particle positions, generate a synthetic image as ground truth (10 points)**

Generate a raw image by using the coordinates of detected particles in the first frame of the image sequence. Convolve the raw image with a Gaussian that approximates the PSF to generate a synthetic image. Further implementation details will be discussed in class.

#### **B.3.2 Implement a sub-pixel resolution detection algorithm using (15 points)**

You can choose to implement either the cross-correlation or the Gaussian fit algorithm in [1]. Quantify detection performance by calculating the mean and standard deviation of detection error by using the synthetic image ground truth generated in B.3.1.

### **C. Report format**

There is no page limit to the report.

Page size: letter  
Line space: single  
Page margins: no less than 1 inch  
Font size: 12 points for the main text; 10 points for listed references

### **D. Submission of MATLAB codes**

This course has its registry in CMU Blackboard (<http://www.cmu.edu/blackboard/>). We will use the “Digital Dropbox” tool for submission of MATLAB codes.

### **Reference**

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