PALM Microscopy

Outline of the project

PALM microscopy consists in estimating the position of fluorophores with subpixel resolution in order to reconstruct images having a resolution better than the diffraction limit.

The goal of the project is

- To study the resolution that can be reached by this method, and the optimal parameters of the imaging system parameter. To simplify, this study is to be done on 1 dimensional signal.

- To implement the algorithm for reconstruction a higher resolution image from a sequence of images of isolated fluorophores, and apply it to a test image.

This work is to be reported under the form of a scientific journal paper with an archive file containing your source codes. To this end, templates of the journal "Optics Express" are posted on Libres Savoirs (in MS Word and Latex), together with an example of Optics Express paper. The length of the paper is limited to 10 pages.

The features that must be reported in this work are:

- Precision analysis (1D):
  - Simple and quick analysis of the noise of the given images, analysis of the CRLB when the parameter a of the PSF is unknown, especially its variation with θ, according to the noise model (previously found).
  - Expression of the ML estimator, and estimation of its bias and efficiency.
  - Optimal value of the PSF width w.

- Implementation of the algorithm (2D)

- Algorithm for detection and rough estimation of the position of single fluorophores in an image.
- Expression and implementation of ML position estimator in 2D.
- Application of the method to the proposed images and find the hidden pattern.

For this project, the PSF is supposed to be an isotropic Gaussian pulse with the same FWHM $w = 2$ in the two directions and maximal value $a = 1$. The given numerical values are dimensionless.

The previous points are only guidelines. You are free to order them as you want. The only requirement is that the "flow" of the paper is logical.

The article is to be handed in PDF format.

Data (available on Libres Savoirs)

- `ImageTest.mat`: reference image for testing your algorithm.
- `CoordinatesTest.mat`: exact positions of the fluorophores in image `ImageTest.mat`.
- `ImagesPALM.mat`: sequence of 865 PALM images.
- `BlurredImage.png`: low resolution image of the pattern to reconstruct from `ImagesPALM.mat` sequence.

Matthieu's tips

1. Try to use elementary functions as best as you can in your code (through the use of Matlab functions rather than long scripts or long lists of commands). Indeed small functions are easier to read and debug.
2. If you use Matlab scripts, do not forget to clear your variables from time to time to avoid issues such as unwanted changes in vector or matrix sizes.
3. In the case of an exhaustive, but coarse, search of multiple local maxima in an image, you can proceed as follows: search for the global maximum, then set the corresponding and surrounding pixels to zero, search for the next maximum, then set its pixel and the surrounding ones to zero and repeat the operation until you have found all the wanted maxima.

4. For a precise (subpixel) estimation of an extremum, try to use the Matlab function `fminsearch` which works fine in 2D...

5. This project implies to process a pretty large amount of images. Therefore, before naively using your code on all the images at once,

    - First, try your algorithm on a single image for which you know the exact location of the fluorophores (for example by using the files `ImageTest.mat` and `CoordinatesTest.mat` which are available on Libres Savoirs)
    - Then, estimate the computation time needed for the whole set of images by calculating it for one image (through the use of the `tic toc` functions for example): It can be interesting to know if you have to let your program run all night long for example...

6. Do not forget to correctly read the Matlab error messages and do not forget that the `doc` and `help` commands give you access to the help instructions of a given function/command.

7. Any questions? Any overwhelming issues? You know how to contact us.