LocalizeJ

IMAGEJ PLUGIN FOR THE SIMULATION OF LOCALIZATION-BASED NANOSCOPY
Abstract

KEY WORDS: Super resolution microscopy, fluorophore localization, multi-user microscopy facility, ImageJ

Within the last decade various approaches have been established for probing biological structures at the nanoscale level. Precise localization of individual fluorophores is one promising approach in order to achieve high spatial resolution. It has been intensively studied in the past that the properties of the fluorophores namely its stochastical blinking behaviour [1] as well as the chosen localization algorithm [2][3] is equally important in order to obtain optimal results. Nevertheless also the density of fluorophores in the biological structure and the presence of (background) noise can massively influence the obtained results. We present an ImageJ plugin which allows the efficient numerical simulation of temporal image stacks resulting from blinking fluorophores seen in a classical diffraction limited imaging setup. The parameters of the detection system [4], the response of the detection system as well as the properties of the fluorophore can be varied [1] allowing the prediction of the achievable resolution and thereby helping to find the right fluorophores and imaging conditions for the experiment. Due to its openness and modularity the plugin can be easily modified and extended. In addition it is well suited to serve as didactical tool in multi user facilities helping to introduce and promote localization based super-resolution nanoscopy.


Installation

• Requirements
  – ImageJ 1.49k
  – Java 1.6.0_24 (64-bit)

• Download
  – LocalizeJ.jar (provide link)
  – Colt.jar (provide link)
  see also: http://dst.lbl.gov/ACSSoftware/colt/index.html

• Copy to Fiji plugins folder

• For ImageJ user, also add imagescience-2.5.0.jar to plugins folder
You will be asked to open an image.
Camera Parameters have to be provided.
Camera response of the input image will be calculated.
Blink Simulator

- You will be asked to specify the parameters of the fluorophore.
- Camera Parameters have to be provided.
- You will be asked to open an image (which will serve as a mask).
- Parameters for the PSF have to be provided.
- Image stack will be calculated.
Parameters fluorophore

Autosave the time stack (path and name have to be entered below). Once activated the stack will not be displayed.

Autosave the compressed version of particle position and state (on, off, bleached) The path and name have to be entered below.

Display the “ground truth image” before PSF convolution.