

# Bio322- Tutorial: 3-Image Analysis and Estimating the Persistence Length of Microtubules

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*Submission in two stages: Section 1 to be uploaded first. Remaining Sections 2 and 3 to be uploaded after the python tutorial.*

## 1 Image Analysis

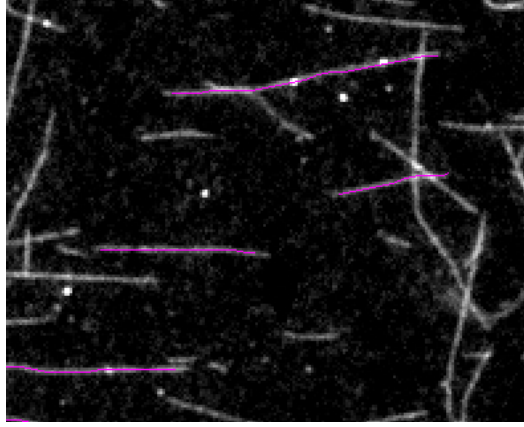
Install the program **ImageJ** on the computer you are using (Win32/64, Lin32/64, Mac OSX).

You will be using only gray-scale images. These have intensity values corresponding to the bit-depth of the image. Do the following:

- Install imageJ
- Find The size of your image in pixel units
- The bit depth
- The minimal, maximal and mean grey value intensity seen in your image
- Plot and save a frequency histogram of your data (report)
- Install the NeuronJ plugin in ImageJ
- Trace  $\sim 10$  fibers
- Save xy coordinates of these as CSV or Tab delimited file
- Upload the coordinates in a single TXT file
- Upload a document describing the image statistics of your dataset

The images provided were generated using goat brain tubulin, polymerization buffer and GTP. These bending seen in the filaments is due to the statistical nature of the polymer subject to thermal noise. We can either study the dynamics of a single filament or the ensemble of many filaments. In this case we use the latter (assuming the system is ergodic).

1. Open the image in ImageJ (**ImageJ website**)- download and install the basic program.
2. Install NeuronJ- a neuron tracing plugin by installing the \*.jar file in the plugins folder of ImageJ (wherever you installed it) and restart ImageJ.
3. Navigate to Plugins – NeuronJ. This loads a special menu. From that menu open a cytoskeleton file.
4. Use the tracing tool to trace out some filaments and save the coordinates of the filament.



(a)

Figure 1: (a) A fluorescence image of fluorescently labelled microtubules assembled *in vitro* from goat brain tubulin acquired on a Nikon inverted microscope (by Kunalika Jain). Scale 1 pixel =  $0.113 \mu m$ . Tracings of individual microtubules are overlaid (colour) obtained using NeuronJ, an ImageJ plugin.

## 2 Introductory python programming

Ideally you should have Python with Numpy and Scipy installed. The simplest distribution is **CanoPy** <https://www.enthought.com/products/canopy/>, since this release by Enthought contains all the packages in one place.

As compare to spreadsheet programs like MS Excel these tools have a better numerical accuracy and are more flexible and extensible. Hence do not use Excel for the next steps, other than possibly for data storage.

A very introductory tutorial will be conducted in class preceding the completion of this part of the assignment.

### 2.1 Reading the data

Read in xy coordinate data using a script described in class and a skeleton script that will be uploaded for you.

### 2.2 Fitting data

Fitting can be done in multiple ways, but the simplest one, **curve\_fit**, is invoked by the following function:

```
>>from scipy.optimize import curve_fit
```

## 3 Estimating the persistence length ( $\xi_p$ ) from correlations

The biopolymers can be considered to be curves in space and they can be characterized by a correlation function. The average directional correlation ( $\cos(\theta)$ ) between two segments of the polymer decreases exponentially with their separation ( $\Delta s$ ):

$$\langle \cos(\theta)(\Delta s) \rangle = \langle \vec{u}(s) \cdot \vec{u}(s') \rangle = e^{-|s'-s|/2 \cdot \xi_p} \quad (1)$$

where  $\vec{u}(s)$  and  $\vec{u}(s')$  are the unit vectors tangent to the chain at positions  $s$  and  $s'$  respectively in a 2D system.

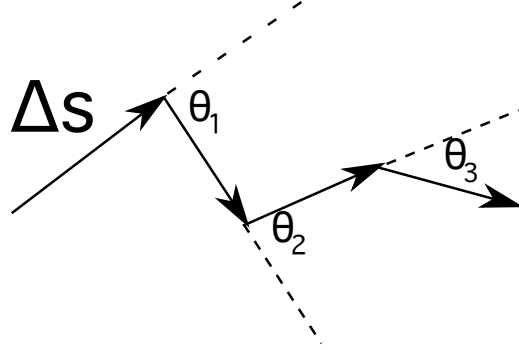


Figure 2: The angles between tangents for a given step size  $\Delta s$ .

- For a given  $\Delta s$  calculate all the  $\cos(\theta)$  values and find a mean. Do this for increasing step sizes.
- The calculate successive  $\cos(\theta)$  angles between each vector (lines in 2D with  $(x1,y1)$ ,  $(x2,y2)$  as start and end) are:  
 Cos theta between two lines using the dot product  
 divided by the product of vector magnitudes  
 $dx1 = x2-x1;$   
 $dy1 = y2-y1;$   
 $dx2 = x4-x3;$   
 $dy2 = y4-y3;$   
  
 $d = dx1 * dx2 + dy1 * dy2$  // dot product of the 2 vectors  
 $l2 = (dx1 * dx1 + dy1 * dy1) * (dx2 * dx2 + dy2 * dy2)$  // product of the squared lengths  
 $\cos(angle) = (d/\sqrt{l2})$
- Plot the  $\langle \cos(\theta) \rangle$  as a function of  $\Delta s$
- Fit an exponential decay. You should be able to extract the factor  $\xi_p$  from the fit.

## 4 Submissions

Submit in two parts as described before:

### 4.1 Image analysis of MT filaments

Section 1 requires you to provide

1. document with the quantification of your image
2. text file with coordinates of the MT filaments

This work will be submitted team-wise. Please include the following in your submitted document: Names of each team member and roll number. Contribution of each team member (e.g.: Sneha analyzed the data, Salim plotted the figures and Ramesh wrote the document).

## 4.2 Data analysis and fitting

This part is also to be continued in the group-work mode. Submit the python code and a document containing:

- the plot of the  $\langle \cos(\theta) \rangle$  as a function of contour distance
- the fit parameter  $\xi_P$
- comparison to published values (discuss the reasons for deviations if any)

The title your submission should be “a003.1-groupnumber-pun.pdf” and “a003.2-groupnumber-pun.pdf” for parts one and two. Do not forget to include ALL names of members of the group and their individual contributions.

Replace **pun** with **tvm** for IISER-Tvm.

Submit via Google-Classroom (pune) and form on the Google-Site. If you face problems, email us at [bio322@students.iiserpune.ac.in](mailto:bio322@students.iiserpune.ac.in).

The title of the email should be “Assignment 3.1: MT Persistence length” or “Assignment 3.2: MT Persistence length”.

## References

- [1] Rivetti et al. (1996) Scanning Force Microscopy of DNA Deposited onto Mica: Equilibration versus Kinetic Trapping Studied by Statistical Polymer Chain Analysis. J. Mol. Biol. 264: 919.
- [2] Kawaguchi and Yamaguchi (2010) Temperature dependence rigidity of non-taxol stabilized single microtubules. Biochem. Biophys. Res. Comm. 402: 66.
- [3] Loong et al. (2012) Persistence length of human cardiac  $\alpha$  tropomyosin measured by single molecule direct probe microscopy. PLoS One.7: e39676.
- [4] Phillips, Kondev and Theriot (2008) Physical Biology of the Cell. Garland Science, New York. Illustr. by Orme.