Bio322- HandsOn-2: Estimating the persistence length of microtubules from contour data

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1   Before you begin

Software: Download and install the program ImageJ on the computer you are using. This will be used to analyze the image.

   You will use Octave to read in, plot and fit data. Avoid the use of spreadsheet programs like MS Excel, it was made for cash management and you have no way of controlling numerical accuracy. Refer to the previous in class tutorial on Octave and the last assignment. For more questions refer to the EXTENSIVE help provided WITH these tools (for octave type $>>help\ function$,
where $function$ is the command you are looking to find out more about).

   Data: 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).

2   Evaluating the bending of cytoskeleton

1. You are provided a text file which has XY coordinates of the traces of Microtubules.

2. Open the image in ImageJ (ImageJ website)- download and install the basic program.

3. Install NeuronJ- a neuron tracing plugin by installing the *.jar file in the plugins folder of ImageJ (wherever you installed it) and restart ImageJ.

4. Navigate to Plugins – NeuronJ. This loads a special menu. From that menu open a cytoskeleton file.

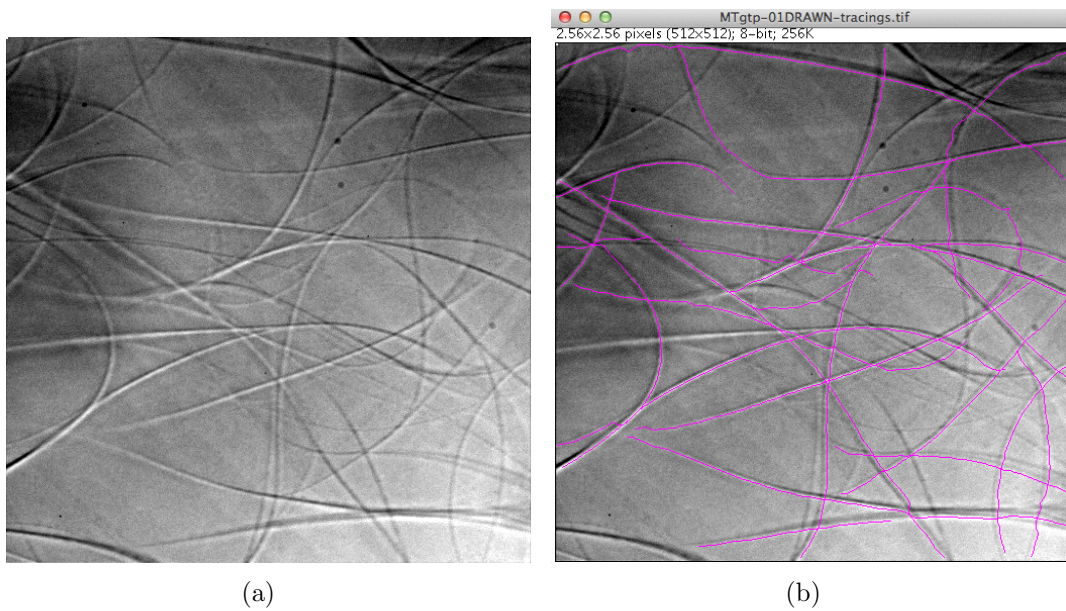5. Use the tracing tool to trace out some filaments and save the coordinates of the filament.

(a)

(b)

Figure 1: (a) A DIC image of microtubules assembled in vitro from goat brain tubulin acquired on an Olympus inverted microscope (Kunalika Jain). Scale 1 pixel = 0.106 μm. (b) Tracings of individual microtubules (and bundles) from an image of *in vitro* assembly of goat brain tubulins in the presence of GTP.

3   Estimating the persistence length (ξ_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 between two segments of the polymer decreases exponentially with their separation:

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

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.
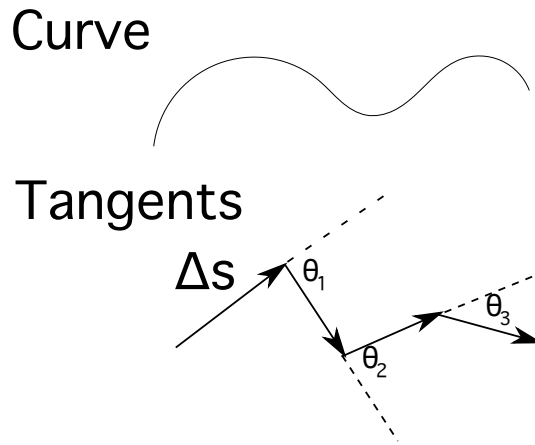

Figure 2: The angles between tangents for a given step size Δs.

- For a given Δs 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 Δs

- Plot such graphs for at least 5 filaments.

- Fit an exponential decay to the combined data. **Octave** has a previously implemented function for exponential fits called **expfit**[5]

- You should be able to extract the factor ξ_p from the fit.

4   Your submission

Submit a single document with the following:

1. plot of the experimental data of mean $\langle \cos(\theta) \rangle$ as a function of Δs (x-axis) as points. Fit and plot an exponential decay of the form y=exp(-x/L) where L is the persistence length.

2. Calculate mean ξ_p value for the microtubule-data.

3. Compare it to published values and discuss (i.e. why they might occur) deviations if any.

4. Include a program or script if you used one in the report.

   The title your submission should be "Lab02-yyyymmdd-Yourname.pdf". Submit to bio322@students.iiserpune.ac.in.
The title of the email should be "Lab 2: MT bending".

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 α 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.

[5] Exponential fit using Prony's method