

# Bio322- Lab2: Estimating the persistence length of microtubules from contour data

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## 1 Before you begin

Install the program **ImageJ** on the computer you are using. Ideally you should have MATLAB/SciLab/PyLab/Octave running on your machine. Use some of the very good tutorials on their website to see how to read in, plot and fit data. As compare to spreadsheet programs like MS Excel these tools have a better numerical accuracy and are more flexible and extensible.

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.

## 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 between two segments of the polymer decreases exponentially with their separation:

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

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.

- 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

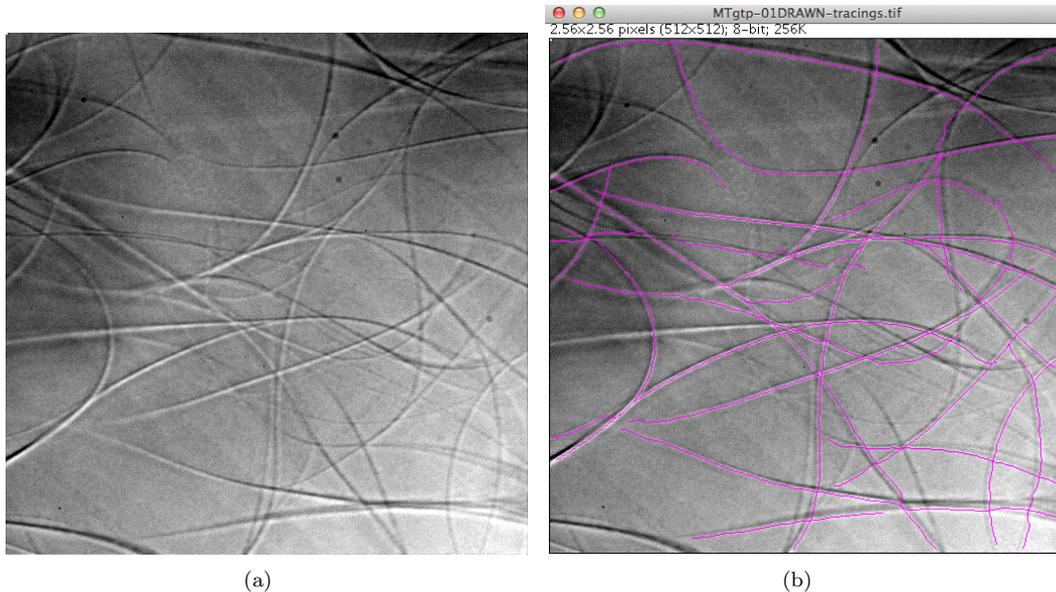


Figure 1: (a) A DIC image of microtubules assembled in vitro from goat brain tubulin acquired on an Olympus inverted microscope (Kunalika Jain). Scale 1 pixel =  $0.106 \mu m$ . (b) Tracings of individual microtubules (and bundles) from an image of *in vitro* assembly of goat brain tubulins in the presence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

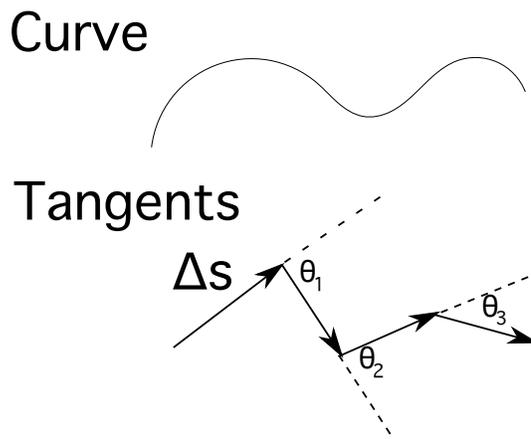


Figure 2: The angles between tangents for a given step size  $\Delta s$ .

```
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 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  $\Delta s$  (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  $\xi_p$  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-yyyyymmdd-Yourname.pdf”. Submit to [comp-celllab@gmail.com](mailto:comp-celllab@gmail.com).**

**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  $\alpha$  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.