

## TubuleJ\_V2

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### Introduction

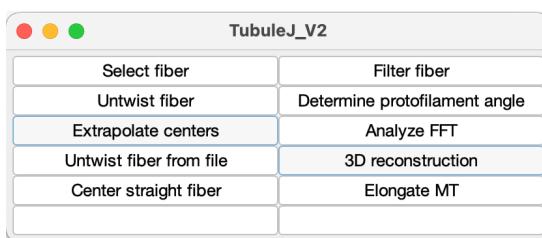
TubuleJ was originally designed to straighten fibers such as microtubules imaged by cryo-electron microscopy (cryo-EM), using the phase information on their  $J_0$  equatorial components in their Fourier Transforms (FFTs). The aim was to obtain sharp diffraction patterns to derive accurate helical parameters on microtubules.

### Installation

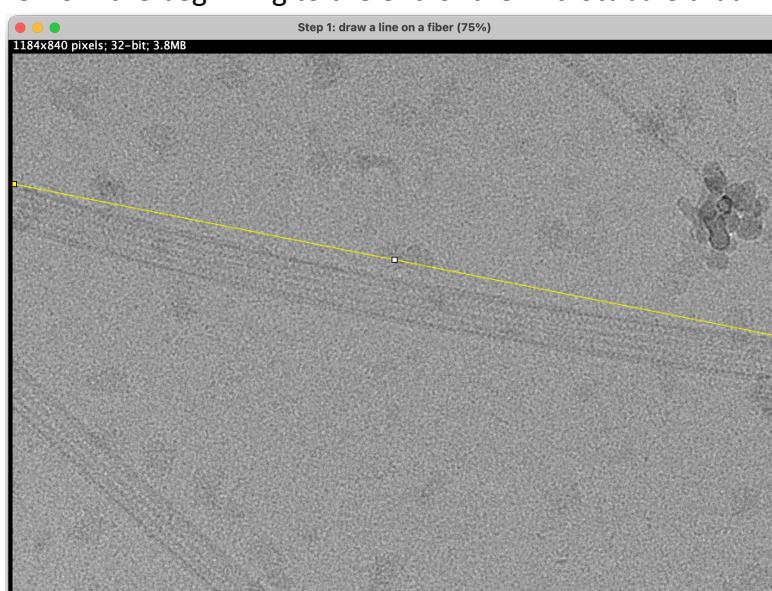
- Download TubuleJ\_.jar and the Tuto.png image at:  
<https://github.com/dchretien35/TubuleJ>
- Copy TubuleJ\_.jar to the plugin folder of ImageJ or Fiji, and create a dedicate folder for the Tuto.png image.

### Procedure

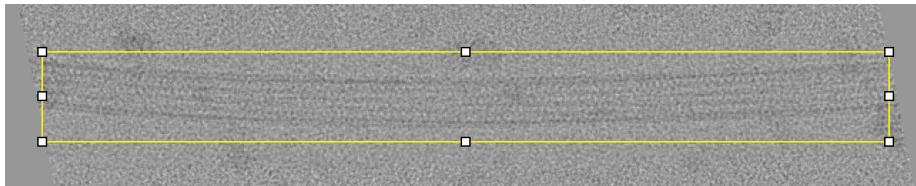
- Start ImageJ/Fiji and open the Tuto.png image.
- Select 'Plugins > TubuleJ > TubuleJ', the interface of TubuleJ opens, together with the 'Log' window where useful information will be provided. Follow also files created in the working folder.



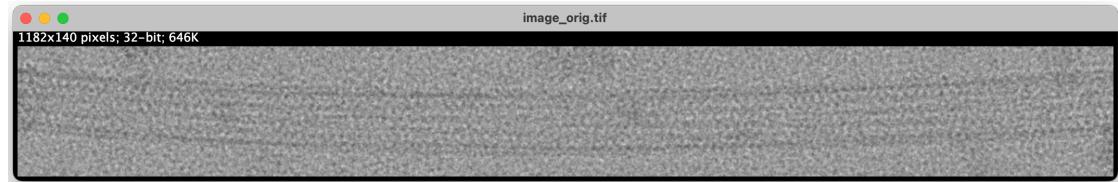
- Click on '**Select fiber**'.
- Create a new folder for the microtubule that will be processed (e.g., Tuto\_MTa).
- In the 'calibration' window, enter the pixel size: 4 Å.
- Draw a line from the beginning to the end of the microtubule that will be extracted.



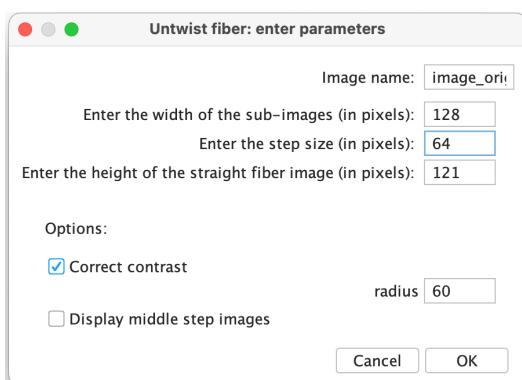
- Draw a rectangle around the microtubule.



The 'Image\_orig.tif' file is presented and saved.



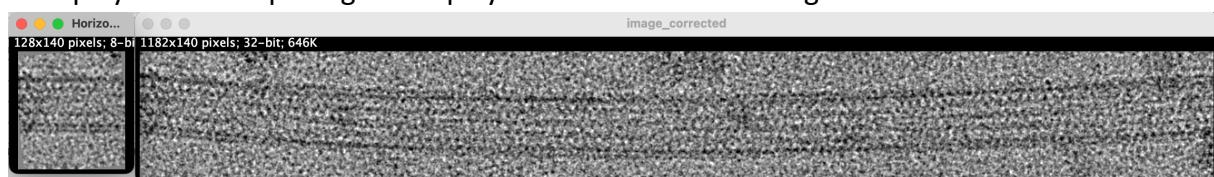
- Click on 'Select fiber'.



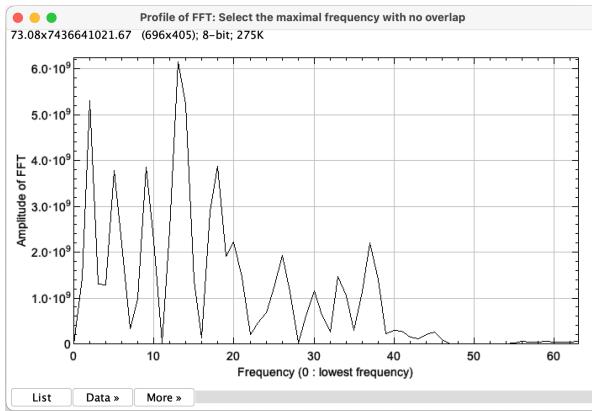
The two important parameters in this window are the 'width of the sub-images' and the 'step size', that is usually fixed as half the image width size. Small boxes provide more local centers, and thus a better description of fiber curvature. However, the straightening algorithm may fail due to a too low signal-to-noise ratio. Experiment different box sizes if necessary. The 'height' of the straight fiber can be let as proposed, since the straightened image will be resized at a later step.

#### *Options:*

- 'Correct contrast': This option (checked by default) allows correction of grey variations in the image, e.g., as a consequence of variations in ice thickness. Let '60' by default unless the 'image\_corrected' still shows contrast variations.
- 'Display middle step images': Displays the extracted sub-images with their local centers.

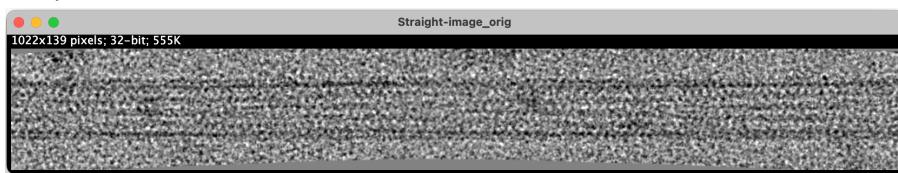


Two images and a plot profile of the equator of the sub-image FFT are presented. On the left: the first sub-image extracted and re-oriented horizontally, and on the right: the contrast-corrected image 'image\_corrected', which is saved and will be used later for 3D reconstruction.



The plot shows a series of peaks. The ones located at low frequency belong to  $J_0$  (Bessel function of order 0, (Diaz et al., 2010)), and will be used to center the microtubule. Those at higher frequencies are usually a mixture of  $J_0$  and  $J_N$ , N=protofilament number.

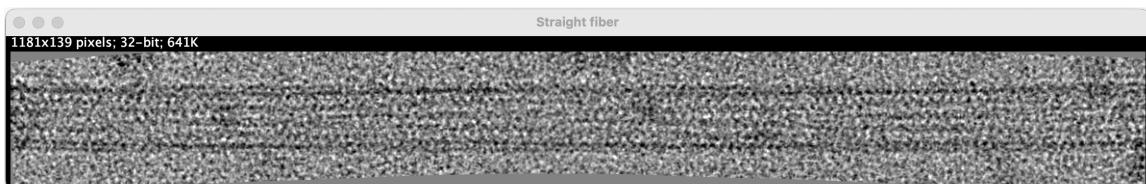
- Click under the third peak from the left (other peaks in this area could also be selected).



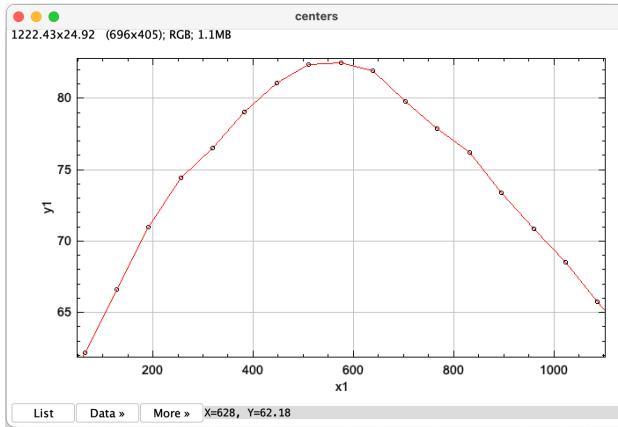
Local centers are determined and listed in the Log window. Centering is based on the analysis of the phases associated with  $J_0$ , which must be 0 (or  $2\pi$ ) when the fiber is centered inside the window (Blestel et al., 2009).

A shorter straightened version of the microtubule is presented and saved as 'Straight\_image.tif'. This image could be further processed for filtration and layer-line analysis, but we may want to process a longer image.

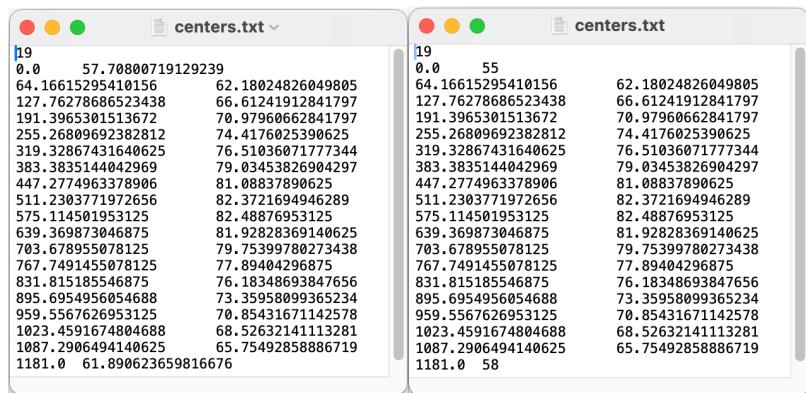
- Click on '**Extrapolate centers**'.



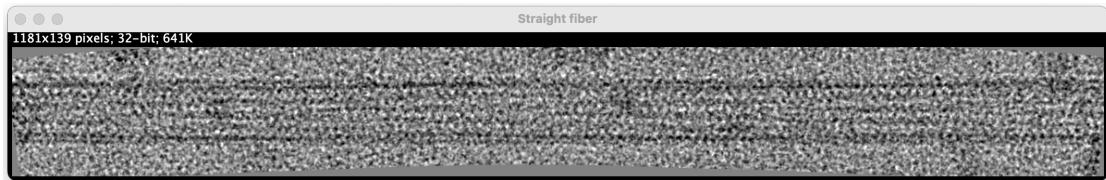
The full-length version of the straightened image is presented, together with a plot of the local centers including those extrapolated at the start and end of the fiber. Here, we see that there remains a slight degree of curvature at both extremities. One possibility would be to straighten again the fiber with smaller boxes. Here, we will edit the 'centers.txt' file and try to correct the local centers manually.



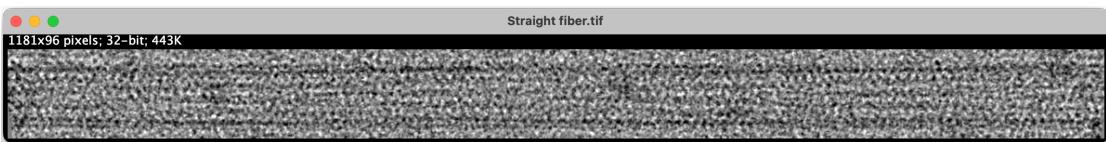
- Open the 'centers.txt' file, change the values of the first and last Y coordinates by a few pixels and save the file.



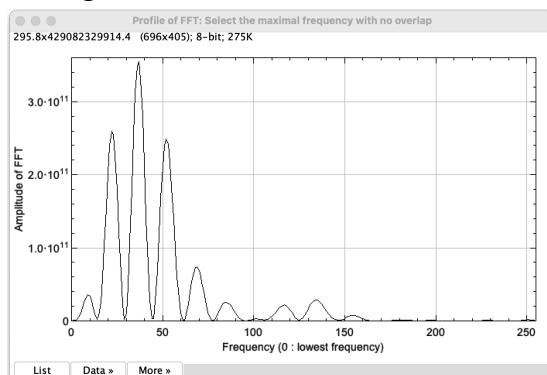
- Click on 'Untwist fiber from file'



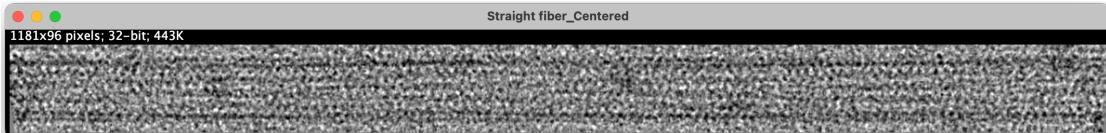
- To improve the signal-to-noise ratio, crop the image to remove some background and save this image.



- Click on 'Center straight fiber'. A FFT size of 1024 x 1024 is proposed, which is close to the length of the image.

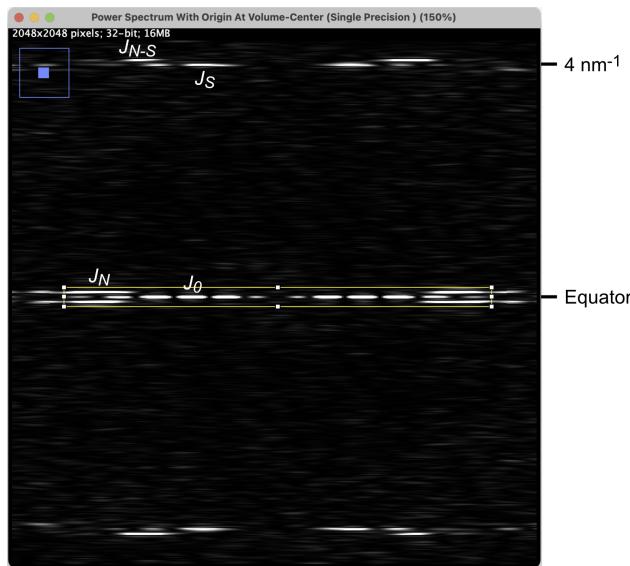


A plot of the equatorial components is plotted as before, but with a clear definition of the  $J_0$  peaks. Click under the second or third large peak from the left.



The image 'Straight fiber\_centered' is presented and saved. The Y translation applied is indicated in the Log window. This image can be further processed for filtering.

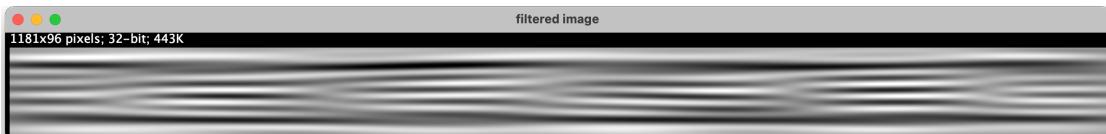
- Click on 'Filter fiber'.



The FFT (power spectrum) of the image is presented. Click on its title, zoom 4 to 5 times (key '+'), and adjust the contrast to enhance the visibility of the layer lines located at  $\sim 4 \text{ nm}^{-1}$ .

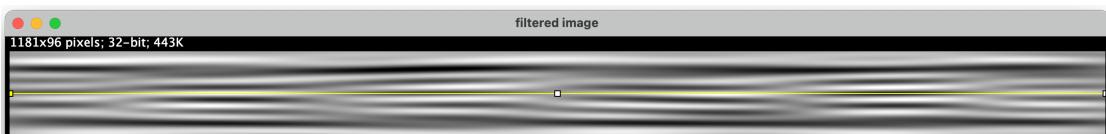
Determine the sign of the protofilament skew angle  $\theta$  from the respective positions of  $J_S$  and  $J_{N-S}$ . It is positive when  $J_S$  is closer to the equator than  $J_{N-S}$ , and negative in the reverse case (Chrétien et al., 1996). Here,  $\theta > 0$ .

Draw a rectangle around  $J_0$  and  $J_N$ , adjust the height of the selection if required, and answer 'Yes' and 'No' to the following questions. Note that additional layer lines could have been selected to filter the microtubule image (Ku et al., 2020).



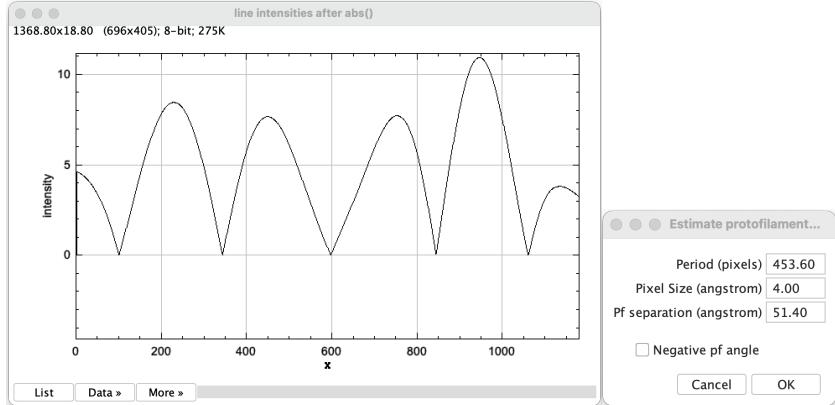
The contrast of the overlapping protofilaments has been enhanced, producing a moiré pattern from which the absolute value of the protofilament skew angle can be determined (Chrétien & Fuller, 2000).

- Click on 'Determine protofilament angle'.



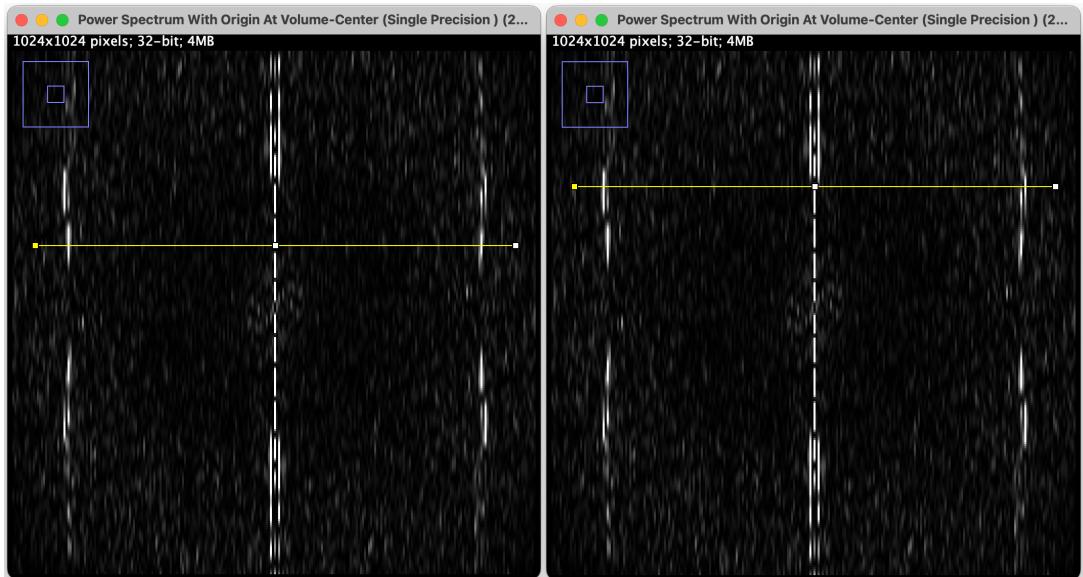
Move the line up (or down) by  $\sim 3$  pixels so that it superposes onto an alternating white and black fringe pattern. This is necessary since the protofilament number is odd (here, N=15). In

the case of an even protofilament number, the line could have been kept in the middle of the image.



Three plots are shown, and a window opens providing the moiré period (in pixels), the pixel size, and the separation between protofilaments (default value of 51.4 Å). The check box is left empty since  $\theta > 0$ . The protofilament skew angle is reported in the log window (theta = 1.62°). We will now determine the repetition between monomer subunits.

- **Click on Analyze FFT.** Zoom and adjust the contrast if necessary to emphasize the  $J_S$  and  $J_{N-S}$  layer lines.

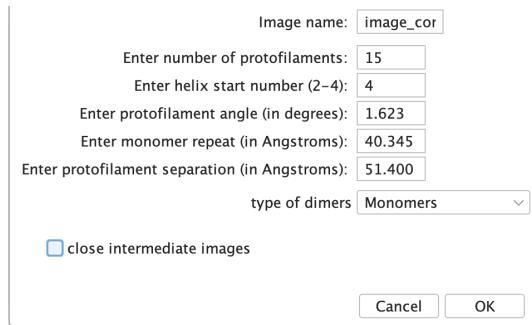


Place the line over  $J_S$  (left), and then  $J_{N-S}$  (right). The plot profiles are presented with a window indicating a search around 40 Å<sup>-1</sup>.

The positions of  $J_S$  and  $J_{N-S}$  are reported in the Log window, as well as the monomer repeat (40.35 Å) corrected for the shift of  $J_S$  induced by the protofilament skew angle.

To check whether the parameters determined and our estimation of  $N$  and  $S$  are correct, we can perform a 3D reconstruction of the microtubule.

- Click on '3D reconstruction'.



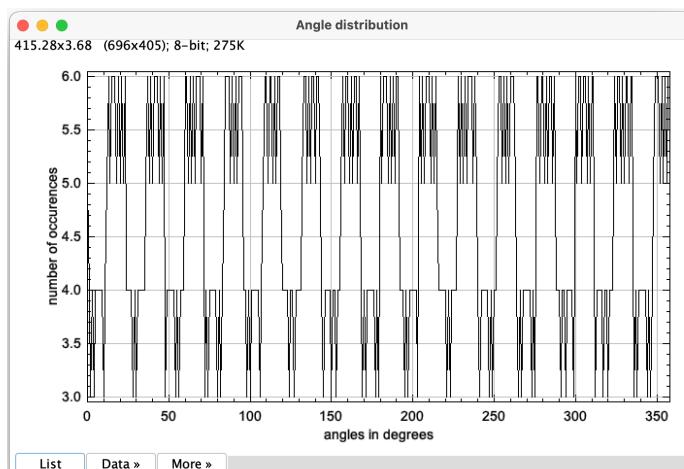
In the window that opens, change the protofilament number to 15 and the helix-start to 4. Keep the 'type of dimers' as 'Monomers'. The 'MT approximate length' is calculated so that it contains at least S-start helices.

*Options:*

'Monomers': sub-images will be extracted at every monomer position.

'Helical dimers': sub-images will be extracted at every dimer position, assuming a B-type lattice organization. Works only for even helical-start numbers.

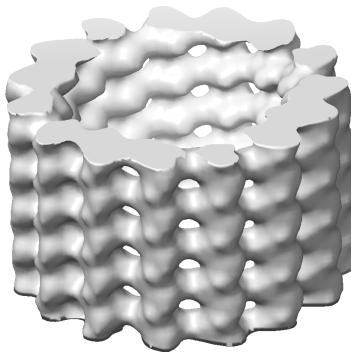
'Non helical dimers': sub-images are extracted every dimers along protofilaments. Highly skewed and long microtubules are required to produce a map devoid of distortions due to missing angles.



The distribution of angles assigned to the sub-images is displayed, which should ideally cover the 0-360° range, which is the case here.

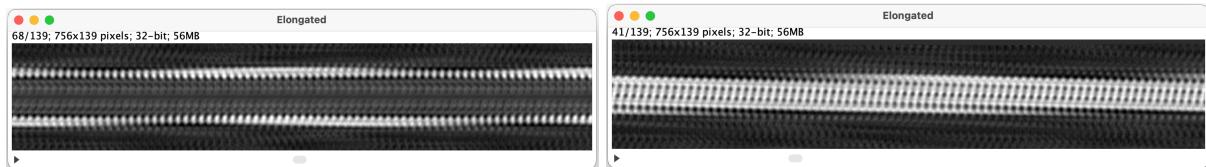


The three stacks presented are the sub-images extracted (left), the same stack with angles assigned to each sub-image (middle), and the 3D reconstruction (right) saved as 'stack\_curved\_3D.tif'. The contrast has been inverted so that it can be directly opened in UCSF ChimeraX for instance:

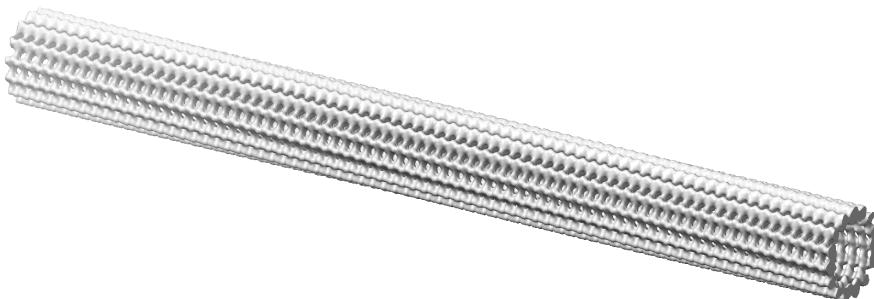


An elongated version of the microtubule can be generated to emphasize the protofilament skew.

- Select the 'stack\_curved\_3D.tif' image, click on 'Elongate 3D MT' and enter a reasonable length (e.g., 756).



This elongated microtubule is saved as Elongated\_3D.tif and can also be visualized in UCSF ChimeraX:



## References

- Blestel, S., Kervrann, C., & Chretien, D. (2009). A Fourier-based method for detecting curved microtubule centers : Application to straightening of cryo-electron microscope images. *2009 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 298-301. <https://doi.org/10.1109/ISBI.2009.5193043>
- Chrétien, D., & Fuller, S. D. (2000). Microtubules switch occasionally into unfavorable configurations during elongation. *Journal of Molecular Biology*, 298(4), Article 4. <https://doi.org/10.1006/jmbi.2000.3696>
- Chrétien, D., Kenney, J. M., Fuller, S. D., & Wade, R. H. (1996). Determination of microtubule polarity by cryo-electron microscopy. *Structure (London, England: 1993)*, 4(9), Article 9. [https://doi.org/10.1016/s0969-2126\(96\)00110-4](https://doi.org/10.1016/s0969-2126(96)00110-4)
- Diaz, R., Rice, W. J., & Stokes, D. L. (2010). Fourier-Bessel reconstruction of helical assemblies. *Methods in Enzymology*, 482, 131-165. [https://doi.org/10.1016/S0076-6879\(10\)82005-1](https://doi.org/10.1016/S0076-6879(10)82005-1)
- Ku, S., Messaoudi, C., Guyomar, C., Kervrann, C., & Chrétien, D. (2020). Determination of Microtubule Lattice Parameters from Cryo-electron Microscope Images Using TubuleJ. *Bio-Protocol*, 10(21), Article 21. <https://doi.org/10.21769/BioProtoc.3814>