**Microtubule Temperature Lab**

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**Purpose:** To observe the effect of temperature on the motion of the Microtubule in terms of the amplitude and the frequency of the microtubule

**Safety:** Wear a lab apron and safety goggles. Be careful with the hotplate and microscope slide

**Testable Question:** How does a change in temperature affect the motility of the microtubule in terms of its Amplitude and Frequency

**Procedure**

1. Prepare Sample
   1. Clean slide with ethanol
   2. Place BSA on glass slides and coverslips to prevent denaturing of protein and prevent it from sticking on the slide
   3. With stuck down microtubules, add another protein called abadin
   4. Prepare dilution
      1. Use buffer GPEM taxol (stabilize microtubule by preventing shrinking)
      2. Warm the buffer with your hand to prevent microtubule from breaking apart
      3. Place .1μl solution of microtubule into 10μl (100x dilution)
      4. Drop 1μl of solution on slide
      5. Put on coverslip on slide
      6. Seal with wax using glass syringe (like a quill)
      7. Label slide
      8. Use immersion oil on slide to prevent diffraction
2. Place the slide of the microtubule in the microtubule and bring it in focus on a microtubule that is stuck down on one end
3. Place the Jump Start temperature control on top of the stage over the microtubule slide
4. Set the temperature of the temperature control at 43°C
5. Record videos of the microtubule at intervals of 2°C until the temperature of 23°C
6. Repeat with ice covering(to see movement at lower temperature than room temp)
7. For each video, skeletonize the image through the following procedure
   1. Choice 1: Use Fiji
      1. Import image as tiff stack
      2. Save original image as
      3. Rotate image if not exactly horizontal
      4. Smooth image using smooth function or do Gaussian Filter - mean filter
      5. Enhance contrast of the image till it is clear
      6. Use Otsu threshold to isolate the microtubule
      7. Skeletonize the image
      8. Invert image
   2. Choice 2: Use JFilament
      1. Import image as tiff stack
      2. Open the JFilament Plugin
      3. Draw the original snake
      4. Track all frames
8. Run the Matlab code as follows
   1. Import Snake.m (from JFilament if used) as a matrix of points
   2. Initialize Parameters of the number of pictures to process using user input
   3. The Image array is then padded with zeros
   4. Combine images to form a “3D” Matrix to process all at once
   5. Filtration occurs in the code
      1. Choose the diameter for the box avg filter (mean filter)
      2. Chose the standard deviation for the a gauss filter (gaussian filter)
      3. Final filtered image is the subtraction of the mean filtered image from the gaussian filter image
      4. Determine if it is necessary to redo the filtering if the result is unsatisfactory
   6. Start doing the Filament Reconstruction Algorithm (description below)
      1. gets x and y points for each snake (microtubule) in each image
      2. use spline to interpolate points (take the trend)
      3. differentiate the spline to get the tangent line
      4. go through each point in the differential to find angle of rotation
   7. Perform Fourier Transform on the Microtubule to determine percentage of each mode
      1. Determine the number of modes to plot to
9. Save the data from each video
   1. Save the two fourier mode plots along with the image
   2. Save the workspace of the microtubule in order to redo the plot if necessary

**Misc Notes**

* fluorescent light bleaches the microtubule and has it fall apart
* Mix microtubules in order to separate them with the micropipette
* Some Motion in the third dimension
* Lense 100x + 1.6x ocular
* 16 bit images grayscale
* Source of error/uncertainty — the length of microtubule makes it more stiff
* Look at effect of Frame Rate on the microtubule

**Data Parameters**

(slide 1)

Video 1 and 2 Data (1 point stuck down):

Concentration of Microtubules: 10x

Temperature: Room Temp (26 degrees celsius)

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 3 data(2 point stuck down)

Temperature: 39.9 degrees celsius

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 4 data (1 point stuck down)

Temperature: 40.6 degrees celsius

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 5 data (1 point stuck down)

Temperature: 38.3 degrees celsius

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 6 data (1 point stuck down)

Temperature: 34.5 degrees celsius

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 7 data (1 point stuck down)

Temperature: 33.1 degrees celsius - 32.5 degrees celsius

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 8 data (1 point stuck down) - fail

Video 9 data (1 point stuck down)

Temperature: 30.1

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

**Slide 2**

Video 10 data (1 point stuck down)

Temperature: 27.5 - 13.6 (changing temp)

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)

Video 12 data (many ice put on )

Temperature: 13.6

Concentration: 1000x

Amount of Hylite: 50%

Amount of Taxol: 10mM (micromolar)