**Stepwise guide to use CHUKNORRIS-master (tomato ver)**

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**To use the CHUKNORRIS package to analyze the kymograph**: [updated Spring 2025]

**Download R:**

Download R (https://cran.rstudio.com/) and R studio (https://posit.co/download/rstudio-desktop/) from the official websites and install it.

Explanation to the pack:

The original github page: <https://github.com/damineli/CHUKNORRIS>

The paper that explains everything about this pack (if you're interested in the logistic & want to trouble shoot something & want to explore more functions of CHUKNORRIS): <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5853864/>

Note from Keyi: Feel free to download the pack that is finetuned a bit by me. To use the code, once downloaded the R, R Studio, and the CHUKNORRIS folder, you can directly open the r file from the CHUKNORRIS > R folder. In the CHUKNORRIS folder, there is a tutorial pdf written by the developers.

In the folder "R", there is a file named as "Ratiokymo… 40xW zoom 2x". This is for analyzing videos that are taken with 40xW objective with 2x zoom-in. The code file can apply to tomato pollen tubes with acquisition settings of 40xW zoom 2x, time interval=3 s. To apply it to Arabidopsis pollen tubes, the very original code developed by Damineli et al. works better. (please read the annotation in the code for more info)

Other than ratiometric kymograph, the CHUKNORRIS pack can analyze other stuff, including normal single-channel kymograph, synchronization analysis, time-series analysis. More info is described in the tutorial and the paper.

Make the kymograph:

1. Follow the kymograph generation process
   1. draw a straight line along the tube to contain all the elongated part
   2. REMEMBER TO SET THE LINE WIDTH TO "1"! Double click the "straight line", and set the line width to 1. DON'T CHECK SPLINE FIT.
   3. click image > stack > reslice. When the window pops out, use default setting (set both output spacing and slice count to be 1)
   4. Check "avoid interpolation"

1. If needed, transform the kymograph (rotate/flip) to make it look like this (Image > transform):

A green line drawing of a graph

AI-generated content may be incorrect.

1. Extra guidance: when selecting the region for kymograph, you can include as little empty region as possible (if you'd like to save negligible amount of space), but remember that the tip in the first frame has to be at least around 4 um long

Turn image into a data matrix

1. click file > save as > text image. Separately save the data from the two channels as two txt files.

Use the code (THIS IS A STEPWISE GUIDE)

1. Don't change the location of the CHUKNORRIS package folder & the location of files in this folder once everything is set up (it's ok to change but the chuk.path variable in the code needs to be changed too). I would put the code into a fixed location (stored on laptop, or flashdrive/hard drive, and whenever I want to use this r code, I plug in the drive).
2. In order to use it, you have to change the folder path and file name IN THE CODE. (copying from the directory in the file explorer and pasting it to designated location in the r code [Windows]/ - right click the file and select "copy path", then pasting it to designated location in the r code [Mac]). The format will look like "D:/folder/folder/folder/"(the capitalized letter = the drive you save the chuknorris folder in) (Windows) or "folder/folder/folder/" (Mac). There has to be a forward slash at the end of the texts.
   1. file input location - in1.path & in2.path
   2. File output location - out.path
   3. File name - fl1.nm & fl2.nm
   4. Layer name - var1.nm & var2.nm (not necessary tho)
3. The image parameters in the code has to be manually changed. For parameters that are not mentioned later, just keep them unchanged.
   1. Measure everything in imageJ first. Draw a random straight line on the kymograph. Use "set scale" to see how long the line is in pixels and microns, and use # microns/# pixel to calculate the "pixel.size" (I calculate it with a calculator and type in manually. Usually for the same magnification the pixel.size remain the same). Needs to be changed when the magnification changes.
   2. For "time.step", change the number in front of "/60". e.g. if the interval of the time series is 3s, then change it to "3/60"
   3. "tip.pixel" needs to be measured in ImageJ and adjusted when the magnification changes.
   4. "avg.width" see the powerpoint.
4. Hit "RUN" to run the code (per line). You can start from specific line by clicking corresponding line of code before RUN.
5. The file will be automatically generated in the designated folder

Common Error notice:

**General note**: if error 2 occurs with other errors, troubleshoot the error 2 first as this is the easiest to deal with, and then run the code (from the top) again. Sometimes after troubleshooting this one, other errors also disappear.

1. "Error in ar.yw.default(x, aic = aic, order.max = order.max, na.action = na.action, :   
    **zero-variance series**" -- possible reason:
2. "Error: Cannot find the connection" -- wrong file directory. Re-check the typed-in file directory (to see if the folder names are correct) or file name at the very beginning. Can also check if the file is at the correct folder.

E.g.

A close-up of a white background

AI-generated content may be incorrect.

Troubleshooting checklist:

**If error notice occurs while running the code**:

1. Check the file name in the r code (check for spellings, especially spaces, dashed lines)
2. Check the spelling of folder directory. Also, every slash has to be a forward slash

[If there's still something wrong --]

1. Open the kymograph in ImageJ. Check the image quality -- the edges of the pollen tube has to be visible in both RFP and GFP channels; check the direction of the kymograph. Only pollen tubes can occur in kymographs. If there are other bright spots that do not belong to the pollen tube (e.g. a pollen grain happens to be in the kymograph), then the code can't tell where the PT tip is.
2. Check the width and length of the GFP & RFP channel kymographs separately to see if they're consistent (sometimes GFP is 120\*60 but somehow the RFP is 120\*59. It's random. To correct this, try generating the kymograph from the video and save it again)

**If the result generated by CHUKNORRIS looks suspicious** (e.g. unchanged GFP/RFP values, GFP/RFP values = 0, …)

1. Go to the file that is named as "RatioKymo…pdf" and check the GFP channel, RFP channel, and the tip location readout (represented by white dots) that are recognized by the r code. If the white dots severely deviate from where the PT tip is supposed to be, then we know that at least certain values are definitely not trustworthy
2. If the GFP and RFP kymographs, read by the code, looks identical, then open the tif format kymograph in ImageJ again and save GFP and RFP channels again. Then run the code again