

KINERROOT USER GUIDE

This is a program for kinematic analysis of root growth. The program runs on Matlab™. For technical details, please refer to the following paper:

Basu, P., et al., *A Novel Image-Analysis Technique for Kinematic Study of Growth and Curvature* Plant Physiol., 2007. **145**(2): p. 305-316.

This user guide explains the operating procedures of KineRoot using a set of example images in the tutorial below. Before you use it for your own work, make sure to work out through the tutorial completely.

Please note that KineRoot is a set of Matlab programs meant for research only. No commercial use is permitted. The success and quality of result from the program will be highly dependent on the quality of the images and the authors are no way responsible for the results.

Installation:

Microsoft Windows: Unzip the file KineRoot.zip and place it in a folder of your choice. Suppose you keep the program in D:\KineRoot folder. To access it from any folder you have to add the path to this folder to Matlab.

Now go to the folder in which Matlab is installed. Suppose Matlab is installed in C:\Program Files\Matlab. Browse to the folder C:\Program Files\Matlab\toolbox\local. Open the file pathdef.m and add the full path to the folder in which KineRoot is saved to this file. If KineRoot was saved in the folder D:\KineRoot add the following line to pathdef.m just before the line `%%% END ENTRIES %%%`

```
'D:\KineRoot\Program;', ...
```

Now save and close pathdef.m. Launch Matlab and type kineroot at the Matlab prompt to start. If the program does not start, check for any syntax mistakes in pathdef.m file.

Linux/Unix: Unzip the KineRoot.zip file to the folder of your choice and add the path to this folder to the environment variable MATLABPATH. Suppose you saved the program in /home/users/someone/KineRoot. If you are a user of C-shell (csh, tcsh) add the following line in your /home/users/someone/.cshrc file

```
setenv MATLABPATH :/home/users/someone/KineRoot/Program:
```

and if you are a user of Bourne Shell add the following two lines to your /home/users/someone/.bashrc file

```
MATLABPATH=:/home/users/someone/KineRoot/Program:  
export MATLABPATH
```

You have to logout and login back to get access to KineRoot from Matlab.

Tutorial: The following tutorial describes one complete kinematic analysis of a growing bean root. Sample images (jpg files) are provided in the Example folder under the KineRoot folder.

Note: Although the program can handle full size images, it is better to crop the original images to a smaller size so that the program can handle the images rapidly. But remember to keep a scale at least in the first image. From this scale you will calibrate your measurements.

KineRoot can handle color or gray scale tif, jpeg, bmp images.

1. To start KineRoot, launch Matlab™, and type “kineroot” in Matlab prompt (without the double quotes) and press enter. It will open a new window (Fig. 1).
2. Click “file template” button and select the first image file fullimage.jpg which has the scale.

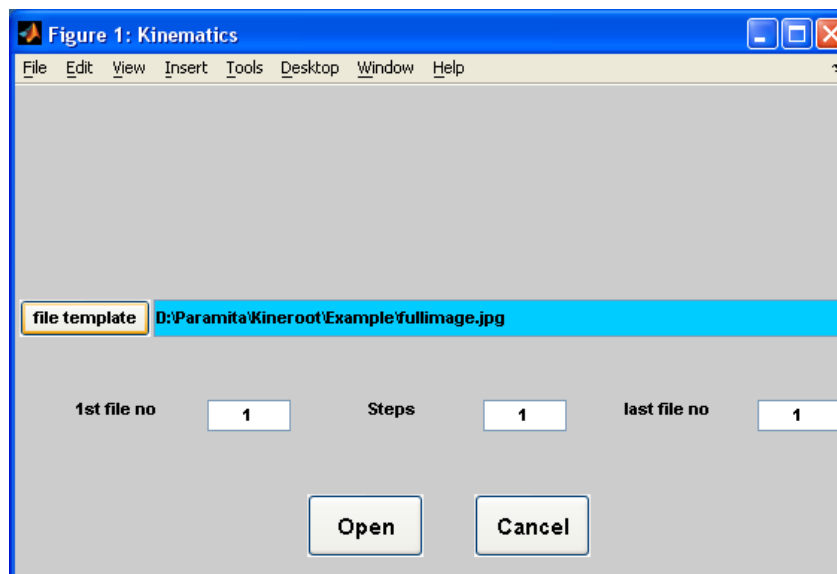


Figure 1

3. After the image is loaded in KineRoot, the main KineRoot window will appear with the image. Note the marks of the ruler at the top (Fig. 2) which are spaced at 1 mm intervals. Click “Measure” at the bottom of the window and then draw a line from any ruler mark to another parallel to the edge of the ruler by holding down the left mouse button (Fig. 2).
4. As soon as you release the left mouse button the length of the line you have just drawn in pixels will appear in purple box below “Scale Info” (Fig. 2). Enter the length of the line in physical units by counting how many mm marks it spans and

enter that in the box left of “pixels=”. Also select the unit of measurement (mm in this case) from the pull down.

5. Type the time interval and time unit between each image frame which would be loaded later. Then click “Ok”. This finishes the spatio-temporal calibration.

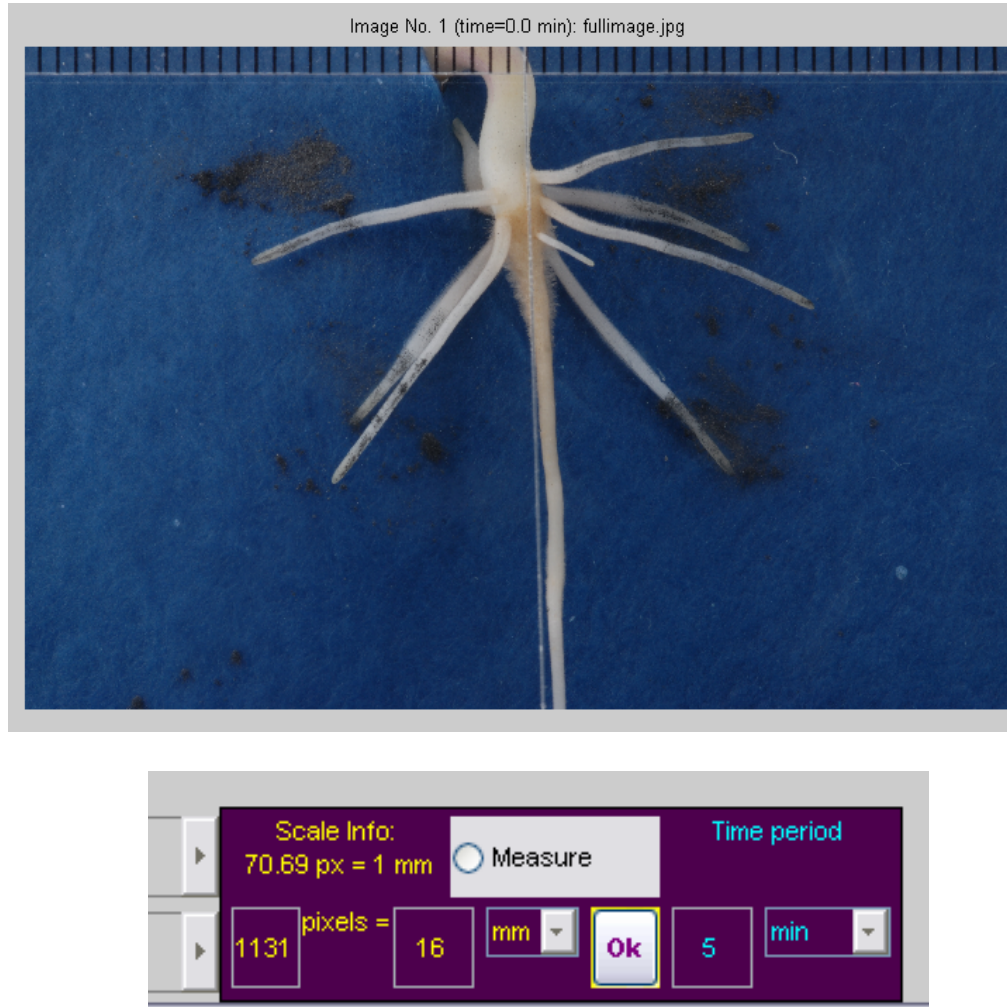


Figure 2

6. Click “Read new set of images” button from the right bottom corner to load the sequence of images to be analyzed. When you click this button, a small window will pop up (figure 3). Click “Yes, but keep the scale” button to maintain the calibration information while loading the image sequence for analysis. Then you will be redirected to image loading window shown in Fig. 1 and 4.



Figure 3

7. For loading multiple image files they must be numbered sequentially e.g. root1.jpg, root2.jpg etc. Select any of the root*.jpg file. Then click on the blue text area to edit the file name. Then we change the name to "...Kineroot\Example\root#.jpg" by putting "#" in place of the sequence number (Fig. 4). (If your files are named root01.jpg and so on, then use ## in place of number, if files are named root0025.jpg, use ##### in place of 0025 and so on.)
8. In "1st file no." box, enter the sequence number of the first file. Here it will be 1 so don't change it (Fig. 4).
9. If you want to skip any files, use steps. By default it will be 1, i.e. every file from first to last will be loaded. Now enter the sequence number of the last file in the last file no. box. Since we have 25 files all of which we want to load, enter 25 and press "Open" (Fig. 4). Please note that if any file is missing an alert will be given on the Matlab prompt.

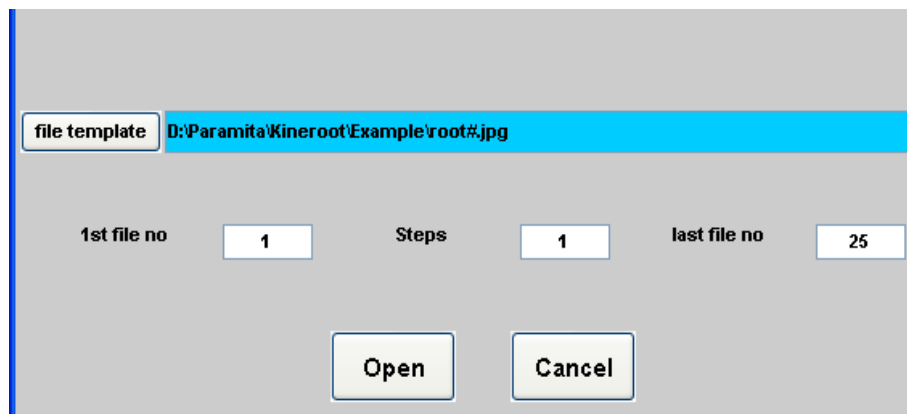


Figure 4

10. Once all the image files are loaded, you can view them as a movie by clicking "Play" button. You can also "Pause" in between image frames. You can also view each frame by frame or even reduce the speed of the movie by sliding the button "0% slower". You can also save what you see in an avi movie by clicking "Save movie" button (Fig. 5).

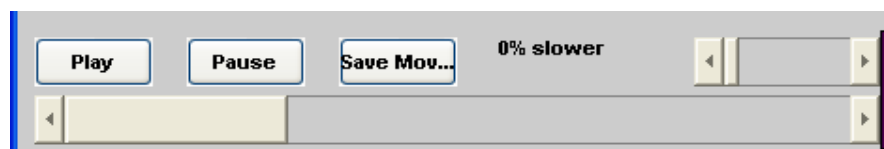


Figure 5

11. For Kinematic analysis you have to select marker points for tracking. You can choose any frame to pick the marker points which will be used as reference. It is

better to pick points in a middle frame because then for tracking both before and after the reference frame, we will remain close enough to the reference which helps any accurate tracking. Use the movie slider (Fig. 5) to go to frame number 12 (say).

12. Now click the “Pick pts” button on the top right corner and click on the image for selecting marker points. Figure 6 is showing picked points in red in image no. 12 from the Example folder. Make sure that you have clicked points either from the root tip or from the base. When you are done selecting points, click right mouse button to stop. You can move any point on the root by clicking “Move pts” and then click and drag the point of your choice. However you cannot remove a particular point.

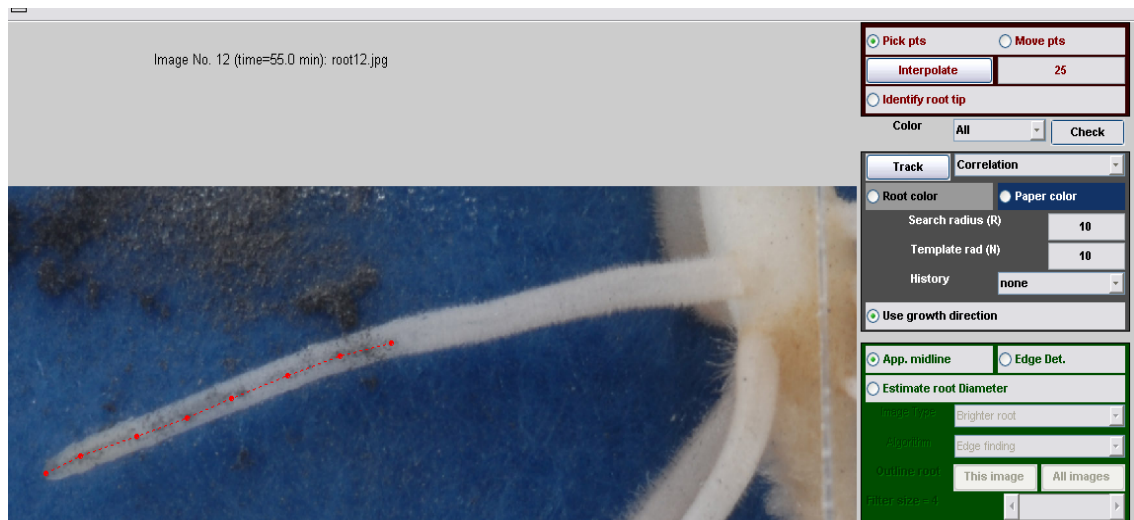


Figure 6

13. You can either select all the points you want to track or increase the number of points by clicking “Interpolate” button. Here the points you added on the root will increase to 25 by default (Fig. 7). But you can change the number “25” to any number you want. Note the more points you track the longer it takes.
14. Before you start tracking, identify the root tip by clicking “Identify root tip” button (Fig. 7) and clicking on the appropriate point on the root image. The root tip will be shown with a cyan ‘*’ symbol (Fig. 9).



Figure 7

15. Since the example image is a color image, you can choose either of red, green or blue color or all the colors for tracking. Tracking works better if you use all the colors. So leave the pull down menu to its default value “All” (Fig. 7).

16. Now we are ready for tracking the marker points on all the frames. Click “Track” to start tracking (Fig. 8).

Note, by default tracking uses “correlation” method. By default, search radius (R) and Template (N) will be 10. No History will be used either by default, so History will be set to “none”. But you can change each of these options. For detailed explanation please refer to the paper by Basu et al., 2007. By default, the button “use growth direction” will also be on (Fig. 8) which means that KineRoot will search for new points by using prediction of velocity. Note, if you want to use color weighted correlation, you have to choose the root and paper color by clicking the appropriate buttons, and then selecting areas on the root and the germination paper respectively.

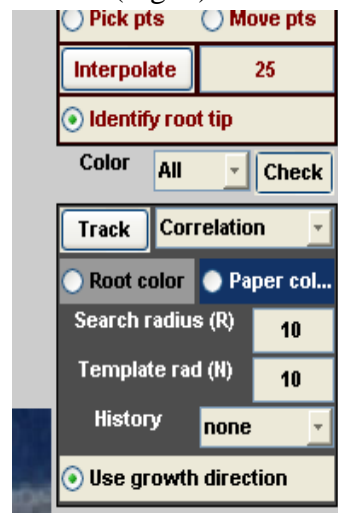


Figure 8

17. After tracking is completed, all the marker points will be placed on all the frames. Play the frames as movie as described in step 10 to see the tracked points in each image. You will also notice a bold white line through the points. This is a least square polynomial fit to the tracked data points (red in color). This line is good enough for measuring root growth, but not sufficient for measuring root curvature (Fig. 9).

18. The button ‘App. Midline’ is on, by default which indicates an approximate midline by using a least square polynomial through the tracked points. But if you need to measure root curvature and root diameter, then you need to click “Edge Det.” button for edge detection. Once this button is on, all other edge detection options will be activated. (Fig. 9). Since edge detection is based on contrast, the color which gives you the best contrast should be chosen. For the example image red color gives the best contrast. Use the color pull down menu (Fig. 8) to choose the color. Click “Check” button to see a gray scale representation of contrast for that color. Clicking “Check” button once more will bring back the original image. For the example image, choose “Red”.



Figure 9

19. For edge detection, you need to provide KineRoot an estimate of the root diameter by clicking “Estimate root diameter” and then drawing an approximate line across the root diameter (Fig. 9). This you have to do only once.
20. Click “This image” (Fig. 9) to detect edge for that particular image only. For your own analysis choose the appropriate image type. There are two algorithms for edge detection. The default method is “Edge finding” which is generally more accurate but time consuming. The other “Gradient finding” method works faster with good quality images. By default, “Filter size” will remain 4. This is the size of the Gaussian filter described in the Basu et al. 2007 paper. You can increase the filter size based on your image quality. Smaller filter size is best for good quality image with low noise, while larger filter size works better with noisy image.
21. After the edges of the root are detected, they will be shown on the image together with the midline (Fig. 10). Please be patient as this process takes a little while. If you are satisfied with the edges detected by the program in the current image frame, click “All images” button, so the program will find the edges in all the image frames (Fig. 10). This step is highly computationally intensive, so the program takes a few minutes to do the job. You can also adjust the root edges manually if necessary by clicking “Manually adjust outline” button and then clicking and dragging the root outline.



Figure 10

22. Once edge detection is completed, again use the play button to see how well the program detected the edges and the midlines. If you are satisfied with the result in every frame then the analysis is complete. Otherwise you play with the filter size for the frame where it failed to detect the root edges accurately or adjust manually.

Please note that the “Display options” allow you to display the different measurements. They have no influence on the actual tracking or edge detection. The buttons are self-explanatory.

You can save measurements by clicking “Save Measurements” button and read those measurements later by clicking “Read Measurements”.

A sample analysis of the example dataset is provided in the file rootanalysis.txt. After all 25 root images are loaded following steps 7-9, you can click the “Read Measurement” file to load this file.

You can reset measurements too by clicking “Reset Measurements” button. Remember there is no undo, so if you don’t save the measurements and reset the measurements there will be no way getting the measurements back.

You can also read new set of images by clicking “read new set of image”.

Visualize results—This button provides basic representation of the data obtained through the analysis. The descriptions of this functionality is provided below.

Analysis of the Measurements: KineRoot provides basic analysis and data visualization capabilities through the button “visualize results”. This will open a new window for presenting the measured data.

In the new window,

1. Click “Plot” from the top menubar and select “Root length map” to get a figure similar to Fig. 7 in Basu et al. paper.
2. Similarly “Plot → Center line growth rate → Raw data” generates growth velocity plot similar to Fig. 8A in Basu et al.
3. “Plot → Center line growth rate → Isocontour” generates isocontour plot of growth velocity similar to Fig. 10.
4. The bottom two menus under “Plot” provide additional control for the plot properties. Rest of the menus are self explanatory.
5. Once a plot is displayed, relevant data for the plot can be saved in tab delimited .txt format for loading in excel by choosing “Save data → Save in text file” or for loading in Tecplot ® (Tecplot Inc. www.amtec.com) using “Save data → Save data for Tecplot”.

Please note that for generating relative elongation rate further post processing of growth velocity data is required which is not part of KineRoot.