Automated Multi-Track Kymography (AMTraK): User Guide

Index

A. Using the executables

The AmtraK algorithm is implemented in MATLAB (R2014b) supplemented with the Image Processing Toolbox and Statistics Toolbox. The executables supplied in combination with the MATLAB Compiler Runtime (MCR) R2014b (distributed freely by MATLAB), allow the user to run the software as a stand-alone application, and no MATLAB installation is required. The user needs to install the MCR using the MCRInstaller file provided.

A.1 Windows

In addition to the AmtraK User Guide, two files have been provided:

- AmtraK.exe (Stand-alone application)
- MCRInstaller.exe (Setup launcher)

On a 64-bit Windows system without MATLAB, the user first needs to install MATLAB Compiler Runtime (version R2014b) using the MCRInstaller file.
Running the AmtraK.exe file displays a GUI as well as a command window. Please refer to Section C for instructions on operating the GUI.

A.2 Linux

In addition to the AmtraK User Guide, three files have been provided:

- AmtraK (Stand-alone application)
- AmtraK.sh (Shell script)
- MCRInstaller (Setup launcher)

On a 64-bit Linux system without MATLAB, the user first needs to install MATLAB Compiler Runtime (version R2014b) using the MCRInstaller file.

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Open the **Terminal** in the directory where the downloaded executables are stored.

Now to run the shell script, type **./run_AmtraK.sh** *space* **path**

where **path** is the location of the directory where the MCR is installed on the machine.

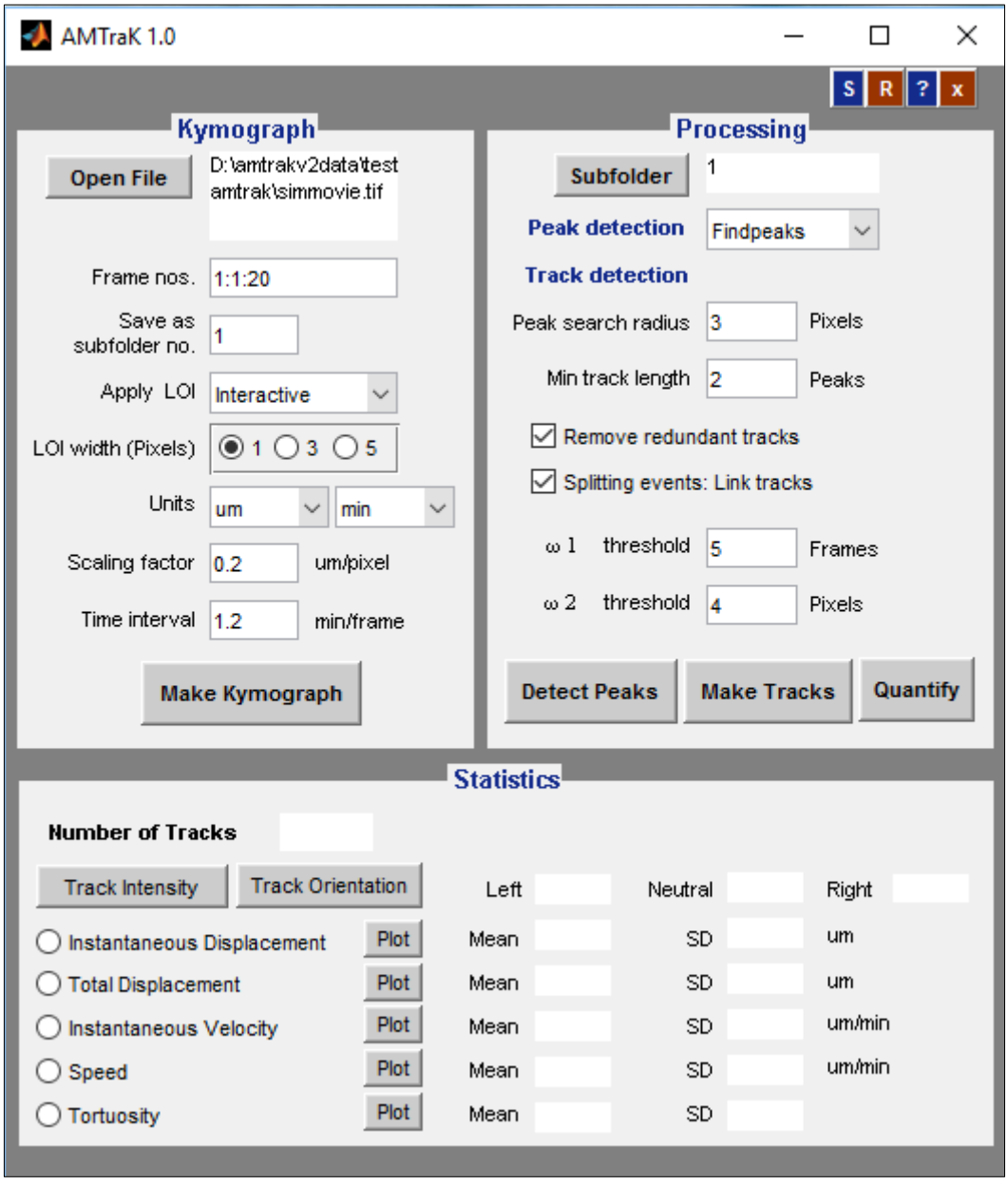
This displays the AmtraK GUI. Please refer to Section C for instructions on operating the GUI.

B. Using the source-code

Back

The AmtraK algorithm is implemented in MATLAB (R2014b) supplemented with the Image Processing Toolbox (ver. 7.3) and the Statistics Toolbox (ver. 7.3). The user needs these or more advanced versions of MATLAB and the toolboxes running on the machine. Invoke the 'AMTraK.m' file in the folder to run the software as a GUI (instructions in Section C).

C. Graphical User Interface (GUI) operation



C.1. Constructing a kymograph

Back

Parameters

File name: Select the image time-series on which kymography is to be performed. Output subfolders will be stored in this parent directory.

The accepted format for the image time-series is given below:

Format	Abbreviation	Extension	Accepted Bit-depth (bits)
Tagged Image File Format	TIFF	.tif	1,8,12,16,24,32,36,48,64

Example- movie1_fl.tif

Frame nos.: It is the range of images in the time-series which are to be analysed. All the frames are selected by default. The number can be edited in the GUI to choose a subset of the series and/or skip frames in between by specifying a step size.

Notation- a: n: b

where a= Start image number

b= End image number

n= Step size

Example-

5:1:20 (All images between 5 and 20, skipping none in between)

1:2:20 (Images between 1 and 20, skipping alternate frames)

Subfolder no. : The output from AmtraK is stored in a subfolder with this index number (amtrak-#). This is especially useful in case the time-series is to be analysed using multiple Lines of Interest and the data from multiple subfolders is to be pooled.

Apply LOI: The kymograph is constructed based on a Line Of Interest (LOI), which can be selected in two ways:

1. **Interactive**

   On clicking the 'Make Kymograph' button, the user is prompted to interactively select a segmented Line of Interest on the maximum-intensity projection image of the image-series, with the help of a mouse. Use normal left clicks to add segments to the line. A shift-, right-, or double-click adds a final point and ends the selection. Pressing Return or Enter ends the line selection without adding a final point. Pressing Backspace or Delete removes the previously selected point from the LOI.

2. **From file**

   On clicking the 'Make Kymograph' button, the user is prompted to select a text file containing coordinates of Line of Interest which can be applied on the maximum-intensity projection image of the image-series. This is especially useful in case of multi-channel images where the same LOI needs to be applied. In this case, the user first needs to generate kymographs for one channel using AMTraK. The LOI coordinate-file is automatically in the subfolder as 'LOIselection.txt'. In order to apply this LOI onto another fluorescence channel, the user needs to first select the new time-series, adjust the subfolder number and relevant parameters and then click 'Make Kymograph'.

LOI width: The width of the LOI in pixels.

Distance and time units: The units in which displacement and time are to be quantified.

Scaling factor: The image scaling factor (distance unit per pixel) from microscopy.

NOTE: Raw microscopy images have implicit scaling factors in μm as units. If the user selects distance units other than μm to make the kymograph, he/she needs to ensure that the scaling factor is adjusted accordingly.

Time interval: The time interval between two consecutive image-frames, in terms of units selected previously.

C.2 Processing a Kymograph

Back

Subfolder: Interactively choose the subfolder (s) to process, named specifically as 'amtrak-#' in the parent directory. In case of multi-subfolder selection, in the dialogue box, choose multiple folders > **Add** > **Done**.

Peak Detection Parameters

Peak detector: The user can select any of the three methods for detecting bright points in the kymograph, namely Findpeaks, Watershed and Canny edge detection.

Tracking Parameters

Back

Peak search radius: The threshold distance value (in pixels) for linking peaks to make a track.

Minimum track length: The minimum number of peaks that a track should contain in order to be considered for analysis. Default value = 2.

Remove redundant tracks: Spurious tracks generated during track-detection are eliminated. It is recommended to keep this option on while making tracks.

Splitting events: (Optional) When checked, tracks within proximity will be linked depending on thresholds ω1 and ω2.

ω1 : A threshold in time (frames) to detect a splitting event.

ω2 : A threshold in space (pixels) to detect a splitting event.

Quantification

Back

The instantaneous and track-wise attributes of particle movement are quantified and can be displayed in the Statistics Panel.

Batch Processing

Back

If multiple subfolders are selected for processing, the user is prompted to fill in the common parameters for processing each kymograph.

The batch-quantification output is stored in a subfolder named 'BatchPro' in the parent folder. The statistics can be displayed in the GUI panel.

C.3 Others

Back

S: (Save Parameters)

When pushed, the button allows input parameters to be saved in a text file in the given subfolder.

R: (Reset)

The parameters are set to blank/default.

D. Output Files

Function	Sr. No.	Output file	Description
Make Kymograph	1	OutputKymo.txt	The kymograph matrix
	2	OutputKymo.tif	The kymograph image
	4	LOIselection.txt	(x,y) Coordinates of Line of Interest
	5	LOIselection.tif	LOI overlaid on projected image-stack
Detect Peaks	6	Peaks.tif	Peaks overlaid on the kymograph
	7	Brightcoords.txt	(x,y) Coordinates of peaks detected in the kymograph
Make Tracks	8	PlotContour.tif	Tracks overlaid on the kymograph
	9	Tracklist.txt	(x,y) Coordinates and intensity of tracks detected in the kymograph
	10	Branchpoints.txt	Intersecting tracks and their branch points
Quantify	11	OutputStats.txt	Summary of particle movement statistics
	12	USER_InstStats.txt	Instantaneous (stepwise) statistics of each track
	13	USER_TrackStats.txt	Averaged statistics of each track
Track Orientation	14	Track_Orientation.txt	Directions indicated by signs. +/- = right/left
	15	Track_Orientation.pdf	Colour-coded tracks overlaid on kymograph, red=right, blue=left, green= neutral
Track Intensity	16	Track_Intensity.pdf	Intensity profiles of each track
Save Parameters	17	All_Parameters.txt	Record of kymograph making and processing parameters

Note: If the initial attempt at building and tracking the kymograph fails, it might have multiple causes. If image-noise is an issue, de-noising the image using a median filter in ImageJ can help. Alternatively, prominent image-drift can also lead to aberrations. We recommend using the ImageJ Stack Registration plugin to correct for translation.