# A short introduction to celltrax pre-processing tools

#### What the app does and what doesn't

The <u>celltrax pre-processing tools</u> Shiny app is an interactive version of two R packages for preprocessing of microscopy cell tracing data: the <u>celltrackR</u> and <u>celltrax</u>, offering basic tools with their most common settings to non-programmers. It enables for correction for cell recognition errors, filtering of the track lengths, gap repair, elimination of cell multiplets, non-specific drift correction and selection of actively motile cells. The user may adjust some of the settings and, finally, download the raw and processed track table along with some diagnostic plots.

Due to limitations of external computation power, the app allows for processing of **a single sample at once with maximal 1500 tracks or 40000 steps** in total. For larger samples, multiple samples or macroscopic object analysis or any highly customized analysis setup, switching to R programming environment is recommended.

#### Importing track data

Raw data in two formats are accepted:

1. Recommended: Single, tab-delimited file with the columns with track **ID**, **time**, **x**, **y and**, **optionally**, **z coordinates** is the preferred format. The program does it's best to assign the column names to the variables, but make sure to control the entries and change them if needed. You may also specify the sample name column: if so, you are asked to select the sample. The program does not support currently multiple samples at once. Note: data processed by the app are returned in the single file format with the naming scheme presented in Figure 1.

id		t	х	У	Z
	1	48	90.8534	65.3943	-6416.8
	1	72	89.5923	64.9042	-6419.93
	1	96	88.6958	67.1125	-6421.8
	1	120	87.3437	68.2392	-6424.08
	1	144	86.274	67.9236	-6425.14
	1	168	84.0549	68.2502	-6426.68
	1	192	85.9669	68.547	-6426.09

*Figure 1:* single tab-delimited text file coordinates

2. Separate files for X, Y and, optionally, Z coordinates each are accepted as well. The first column must define the time variable, the remaining must be named after the track IDs.

Absolute time	Trace 617	Trace 624	Trace 742	Trace 578	Trace 641	Trace 525
0				-41175.7375		-41138.85
1			-41423.55	-41173.95		-41137.7125
2			-41420.1375	-41168.1		-41132.5125
3			-41416.4	-41166.3125		-41136.9
4			-41413.6375	-41164.2		-41126.0125
5			-41411.3625	-41158.8375		-41122.6
6			-41409.7375	-41159		-41127.6375
7			-41408.1125	-41155.9125		-41126.0125
8	-41208.5625		-41412.6625	-41155.1		-41120.975
9	-41207.2625		-41411.525	-41153.8		-41120.325

*Figure 2:* a tab-delimited file containing *X* coordinates

### **Analysis and results**

The data processing is started by pressing the 'Launch' button. In case of any errors, you may simply re-fresh the page by pressing F5. You may adjust the setting for each pre-processing task at the corresponding tabs. The default settings are supposed to work fine for most input human cell samples scaled in  $\mu$ m – please check out the output units in your sample!

Especially at adjusting non-specific drift or selecting actively motile cells, be sure to include proper controls in your experiment. The drift adjustment and motile cell selection are omitted during pre-processing by default and require user's action.

The processed or raw data may be downloaded as tab-delimited text files in the recommended format (Figure 1) with some diagnostic plots of cell tracks and displacement vectors in .pdf format.

## **New analysis**

To start a new analysis, refresh the page (F5) or click the 'Reset form' button in the side panel.