Integrated BioDiagnostics

http://www.ibidi.de/

Chemotaxis and Migration Tool Version 1.01

Visualization and data analysis of chemotaxis and migration processes based on ImageJ

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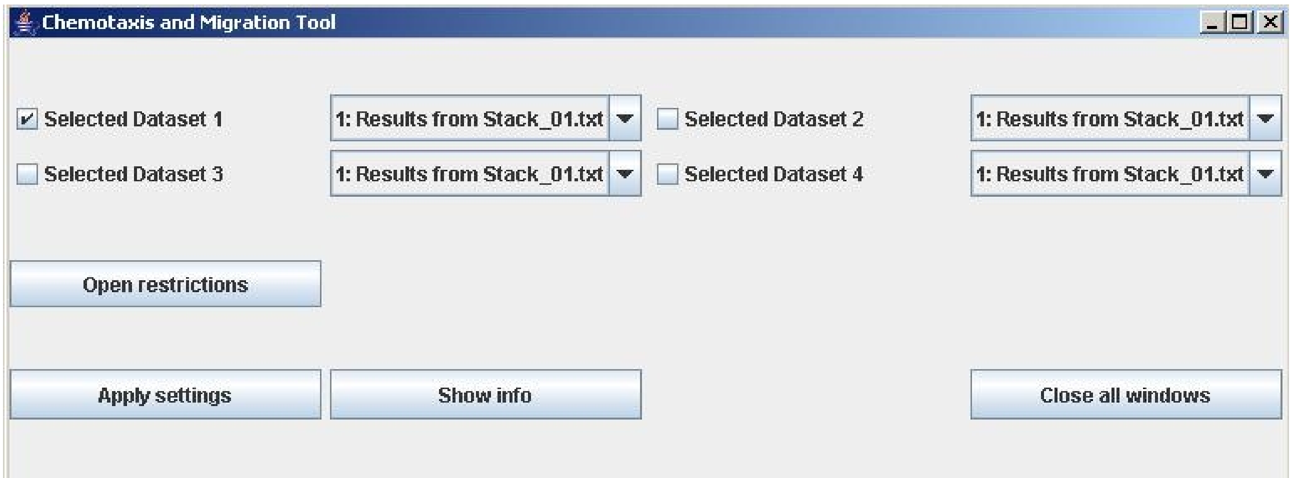
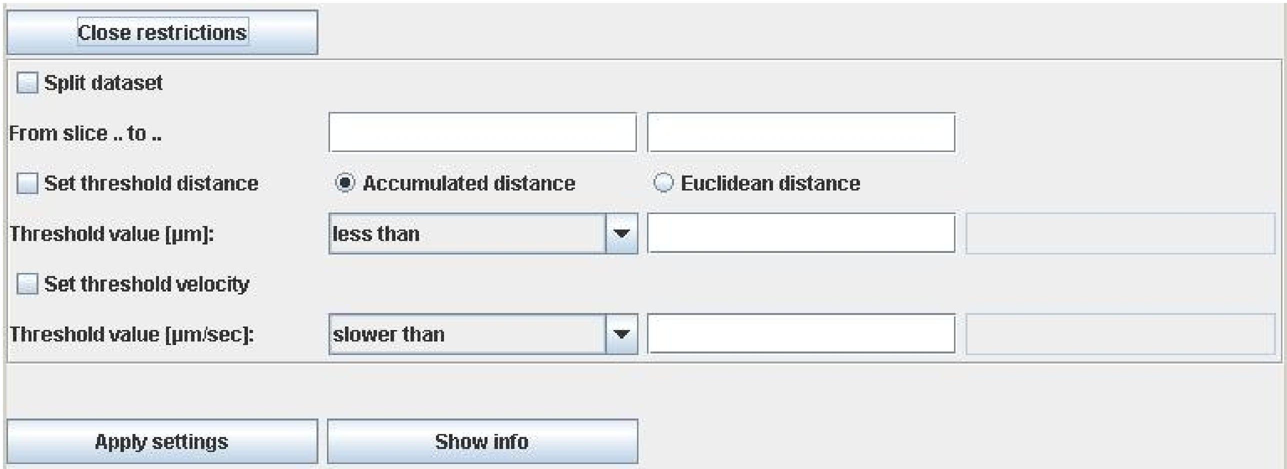


☑ Selected Dataset 1: 1: Results from Stack_01.txt
☐ Selected Dataset 2: 1: Results from Stack_01.txt
☐ Selected Dataset 3: 1: Results from Stack_01.txt
☐ Selected Dataset 4: 1: Results from Stack_01.txt

Figure 1: Main panel

Main panel

Selected dataset:	Provides the opportunity to select up to 4 datasets and work simultaneously on these. Selecting the same dataset more than once is not possible. All functions are applied on all selected datasets, so all datasets can easily be compared to each other.
Open restrictions:	Opens a menu to adjust different kinds of threshold functions. This functions are applied on all selected datasets. See *Restrictions*
Apply settings:	Applies the current settings. If the settings are altered they must always be confirmed with *Apply settings*.
Show info:	Shows important information about the selected datasets. See *Figure 18*
Close all windows:	Closes all open windows.


Close restrictions

☐ Split dataset

From slice .. to ..

☐ Set threshold distance ● Accumulated distance ○ Euclidean distance

Threshold value [µm]: less than

☐ Set threshold velocity

Threshold value [µm/sec]: slower than

Apply settings Show info

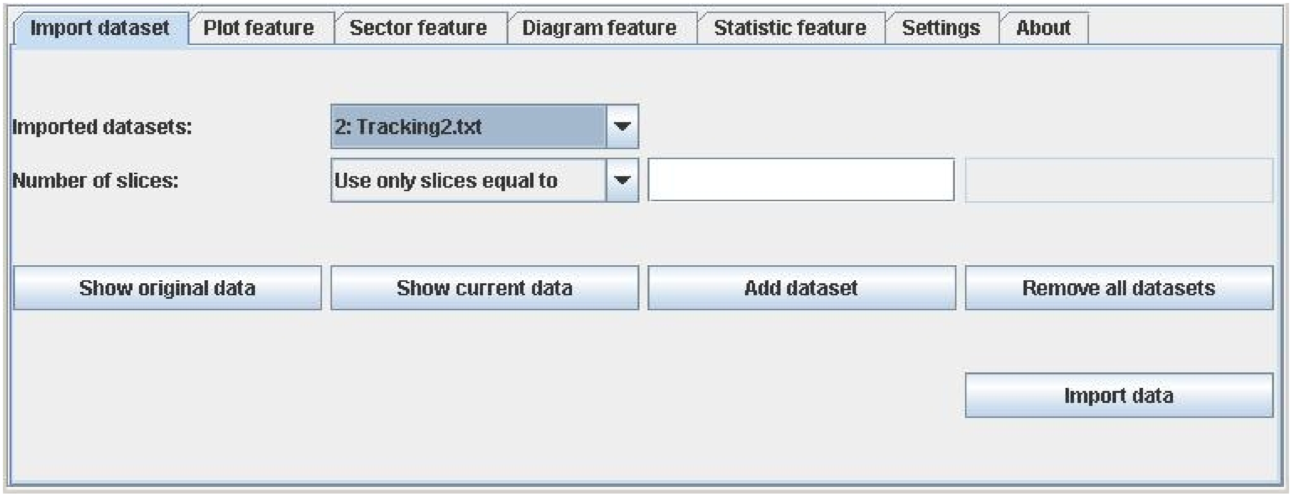
Figure 2: Restrictions

Restrictions

Split dataset:	Splits up the dataset from slice .. to ... Only slices in the specified range are taken into account. For example if you select from slice 2 to 20 (in a dataset of 60 slices) your new dataset consists of 19 slices.
Set threshold distance:	Only tracks whose paths fulfill the adjusted settings are taken into account. You can choose between euclidean and accumulated distance. See *Figure 15*
Set threshold velocity:	Only tracks, whose velocities fulfill the adjusted settings, are taken into account.

To find out reasonable values use *Show Info.*
To compute the velocity and path of each track correctly, the right settings have to be entered at the *Settings menu*.

If you change any settings you have to confirm these by *Apply settings.*



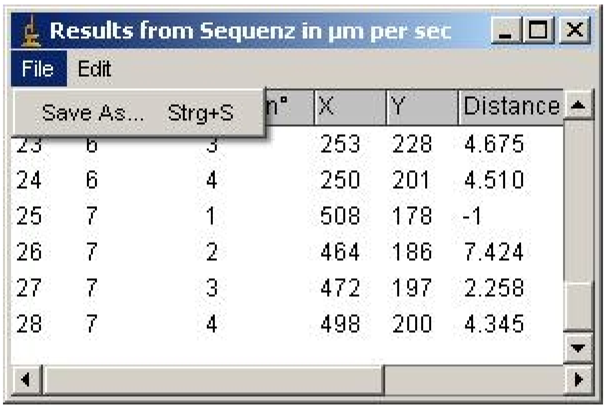
Imported datasets: 2: Tracking2.txt

Number of slices: Use only slices equal to

Figure 3: Import dataset

Import dataset

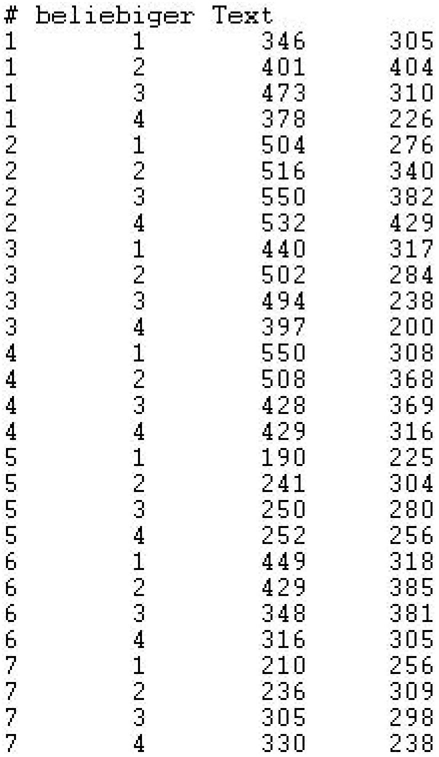
Imported datasets:	Shows all imported datasets.
Number of slices:	Use only slices equal to: Only tracks with exactly this number of slices are used. Tracks with more or less slices are not used. Use slices range from to: Only tracks with number of slices within the entered range are used. See *Figure 6*
Show original data:	Shows the original data for the selected dataset.
Show current data:	Shows the current used data for the selected dataset.
Add dataset:	Adds the selected dataset to *Selected dataset*. See *Main panel*.
Remove all datasets:	Removes all imported datasets.
Import data:	Imports a new dataset. Datasets obtained from the *Manual Tracking Plugin* can be imported directly. (http://rsb.info.nih.gov/ij/plugins/manual-tracking.html), See *Figure 4*



		n°	X	Y	Distance
23	6	3	253	228	4.675
24	6	4	250	201	4.510
25	7	1	508	178	-1
26	7	2	464	186	7.424
27	7	3	472	197	2.258
28	7	4	498	200	4.345

Figure 4: Results table Manual Tracking

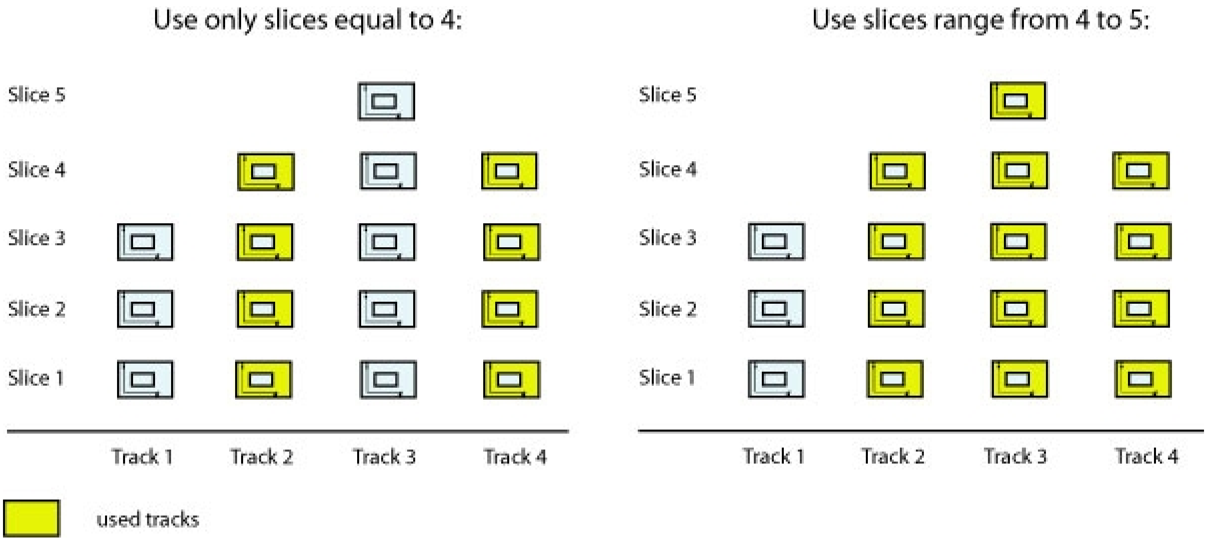
Data from other applications have to be converted to the following format in order to be imported.



```
# beliebiger Text
1       1       346     305
1       2       401     404
1       3       473     310
1       4       378     226
2       1       504     276
2       2       516     340
2       3       550     382
2       4       532     429
3       1       440     317
3       2       502     284
3       3       494     238
3       4       397     200
4       1       550     308
4       2       508     368
4       3       428     369
4       4       429     316
5       1       190     225
5       2       241     304
5       3       250     280
5       4       252     256
6       1       449     318
6       2       429     385
6       3       348     381
6       4       316     305
7       1       210     256
7       2       236     309
7       3       305     298
7       4       330     238
```

Figure 5: Accepted format

The first line is necessary but can contain arbitrary characters.
All following lines must have the following tab separated form.
\t**TrackNumber**\t**SliceNumber**\t**X-Value**\t**Y-Value**



Use only slices equal to 4:

Use slices range from 4 to 5:

Figure 6: Option slices

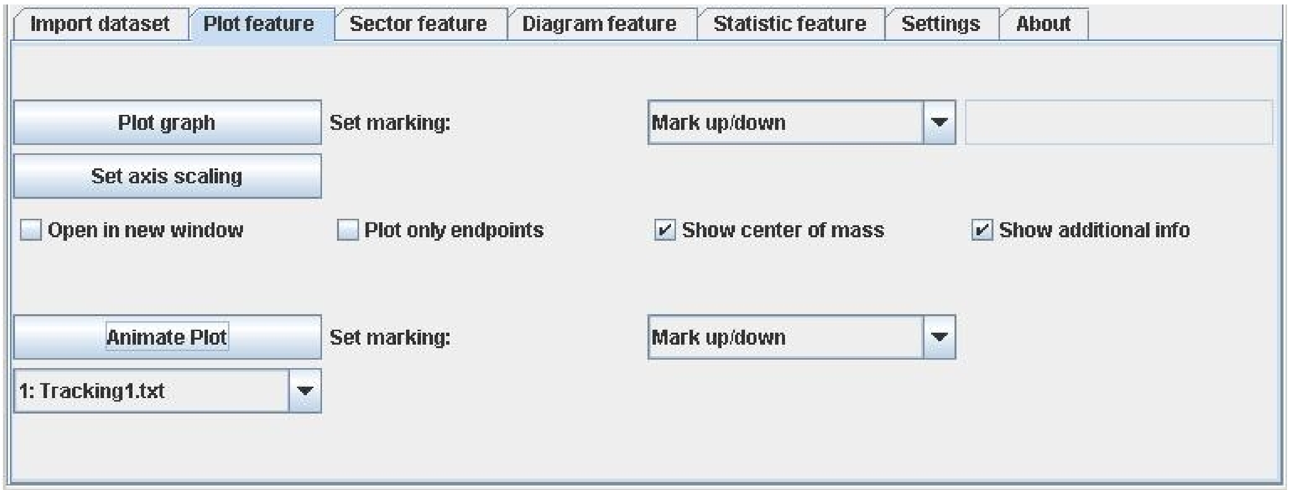


Figure 7: Plot feature

Plot feature

Plot graph:	Draws the plot for the selected dataset. If more than one dataset is selected, more than one plot is opened.
Set marking:	Marks the plot according to the settings.

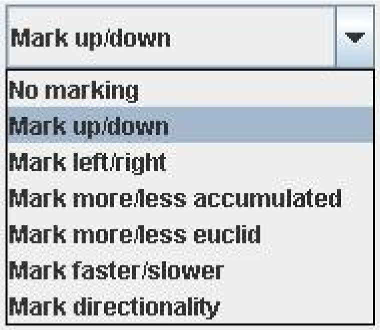


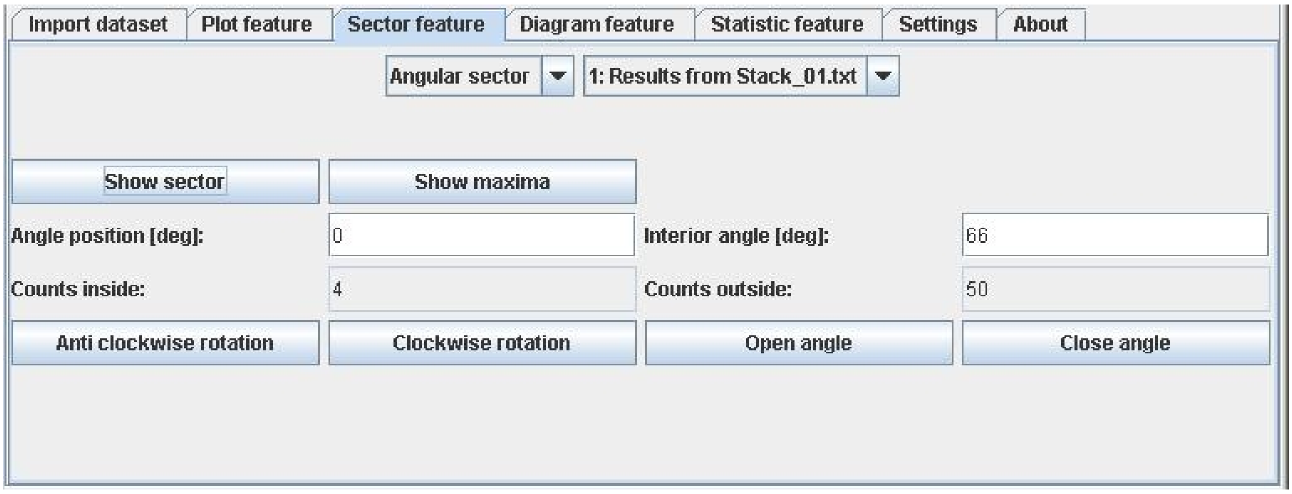
Figure 8: Set marking

Mark more/less, *Mark faster/slower* and *Mark directionality* require the input of a threshold value.

Set axis scaling:	Opens a window to choose the scaling of the plot. The scaling can be set automatically or manually.
Open in new window:	If activated new windows will be opened for each plot. Otherwise the new plot will replace the old windows.
Plot only endpoints:	Shows only the endpoints of the trajectories.
Show center of mass:	Shows the center of mass in the plot. See *Definitions*

Show additional info:	Shows additional information in the plot.
Animate plot:	Chronology animation of the plot. To start the animation select *Image->Stacks->Start animation* in the ImageJ menu. To convert the sequence to an avi file select *File->Save as->avi*. The marking of the the plot is adjusted to *Up/Down*.
Set marking:	Marks the animation according to the settings.

Sector feature



Angular sector
1: Results from Stack_01.txt
Angle position [deg]: 0
Interior angle [deg]: 66
Counts inside: 4
Counts outside: 50

Figure 9: Angular sector

Angular sector

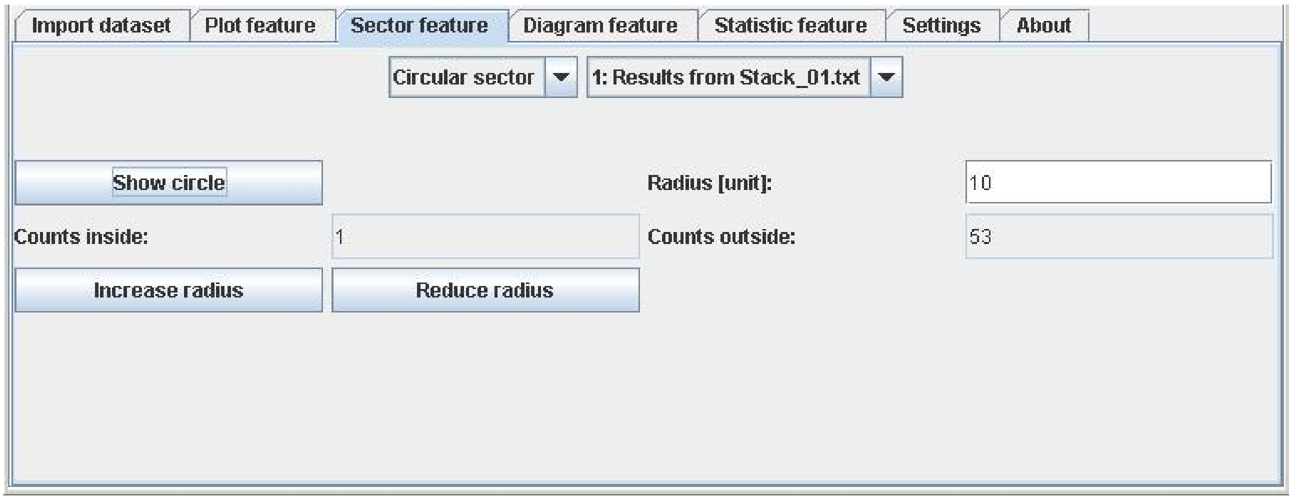
Show sector:	Draws an angular sector field inside the plot. See *Figure 20.*
Show maxima:	Searches for maxima of endpoints within the boundary. If there is more than one maximum you can easily switch between them by clicking the *Show maxima* button again.
Angle position:	Position of the angular sector field. See *Definitions Figure 17*
Interior angle:	Interior angle of the angular sector field.
Counts inside:	Number of endpoints inside the angular sector field.
Counts outside:	Number of endpoints outside the angular sector field.

Note that the endpoints of the trajectories are used for the computation.

Anti clockwise rotation:	Rotates the angular sector field anti clockwise.
Clockwise rotation:	Rotates the angular sector field clockwise.

Open angle: Increases the interior angle of the angular sector field.

Close angle: Decreases the interior angle of the angular sector field.



Circular sector

1: Results from Stack_01.txt

Radius [unit]: 10

Counts inside: 1

Counts outside: 53

Figure 10: Circular sector

Circular sector

Show circle: Draws a circular sector field inside the plot.
See *Figure 20.*

Radius: Radius of the circular sector field.

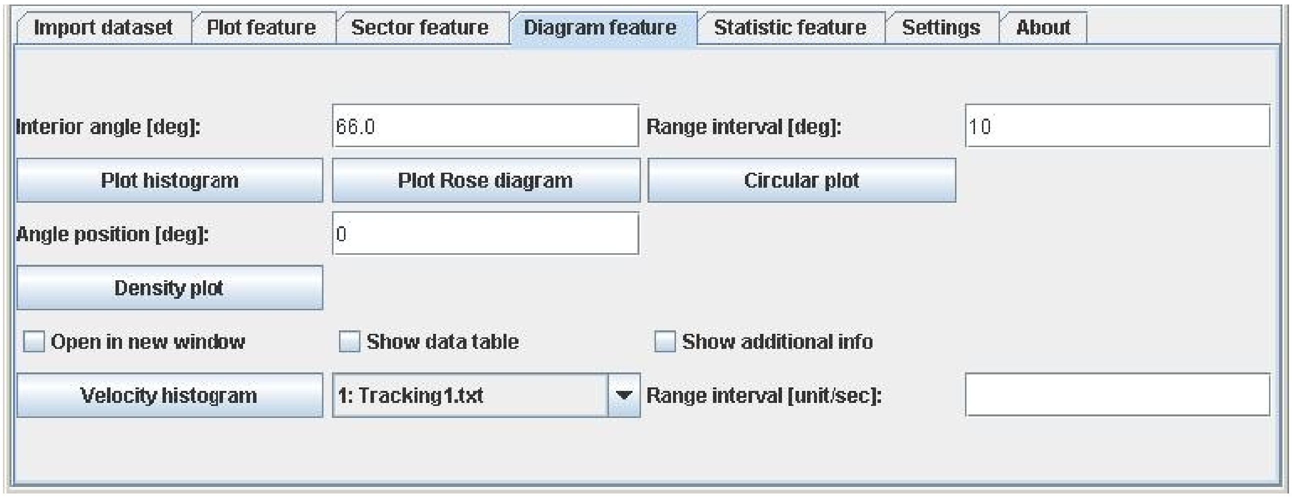
Counts inside: Number of endpoints inside the circular sector field.

Counts outside: Number of endpoints outside the circular sector field.

Note that the endpoints of the trajectories are used for the computation.

Increase radius: Increases the radius of the circular sector field.

Reduce radius: Reduces the radius of the circular sector field.



Interior angle [deg]: 66.0
Range interval [deg]: 10
Angle position [deg]: 0
☐ Open in new window
☐ Show data table
☐ Show additional info
1: Tracking1.txt
Range interval [unit/sec]:

Figure 11: Diagram feature

Diagram feature

Interior angle:	This input field refers to the following plots (*Histogram*, *Circular plot* and *Rose diagram*) and holds the used interior angle of the angular sector field. See *Figure 17*.
Range interval:	Size of the interval for *Rose Diagram* and *Histogram*. The entered value must be an integer. 360 must be divisible by it without remainder.
Plot histogram:	Histogram plot. See *Figure 23*
Circular plot [1]:	Circular plot. See *Figure 21*
Plot Rose Diagram [1]:	Rose Diagram plot. See *Figure 22*
Angle position:	Position of the angular sector field for the *Density Plot*.
Density plot:	*Density Plot*. The plot shows a distribution of *Counts inside the sector / all counts* over an increasing interior angle of the angular sector field. See *Figure 24*
Open in new window:	If activated new windows will be opened for each plot. Otherwise the new plot will replace the old one.
Show data table:	Shows the data for each diagram in a separated window.
Show additional info:	Shows additional information in the plot. For *Density Plot* there is no additional information.

Velocity histogram:	Histogram of the velocities of the objects.
Range interval:	Size of the interval for the *Velocity histogram*. *Show Info* shows the maximum and minimum velocity. With this two values the velocity range can be computed. (*Max Velocity – Min Velocity*). The entered value must be divisible by the velocity range without remainder.

Statistic feature

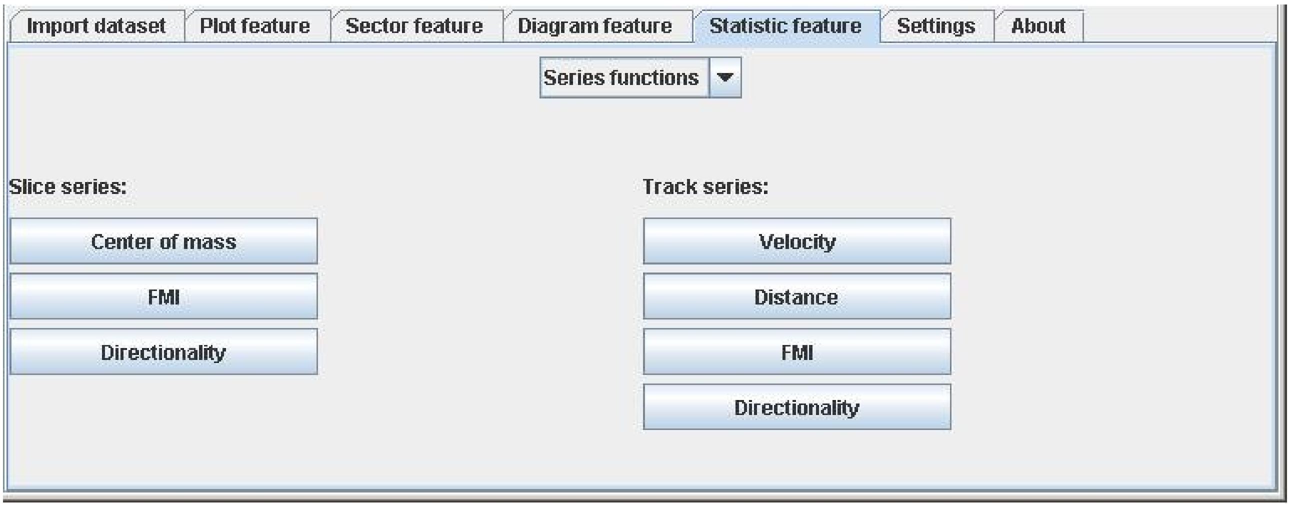


Figure 12: Statistic feature

Series functions

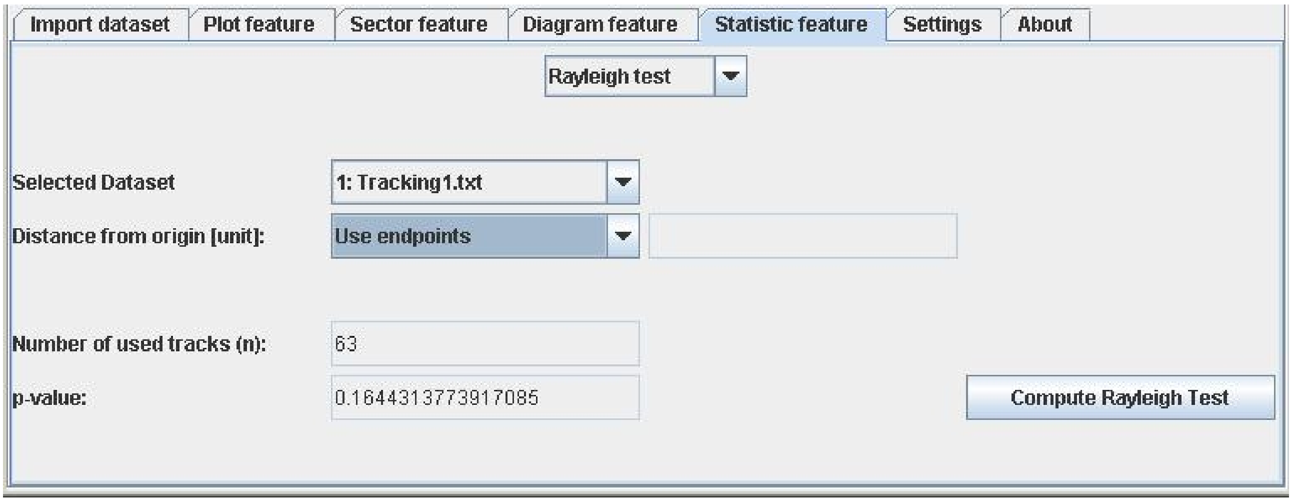
(See Definitions)

Slice series:
Time dependent values for the currently used slices.

Center of mass:	Development of the center of mass over the whole dataset.
FMI:	Development of the *Forward Migration Index* over the whole dataset.
Directionality:	Development of the *Directionality* over the whole dataset.

Track series:
Shows the values for each track.

Velocity:	Velocity for each track.
Distance:	Distance for each track.
FMI:	*Forward Migration Index* for each track.
Directionality:	*Directionality* for each track.



Rayleigh test

Selected Dataset: 1: Tracking1.txt

Distance from origin [unit]: Use endpoints

Number of used tracks (n): 63

p-value: 0.1644313773917085

Figure 13: Rayleigh Test

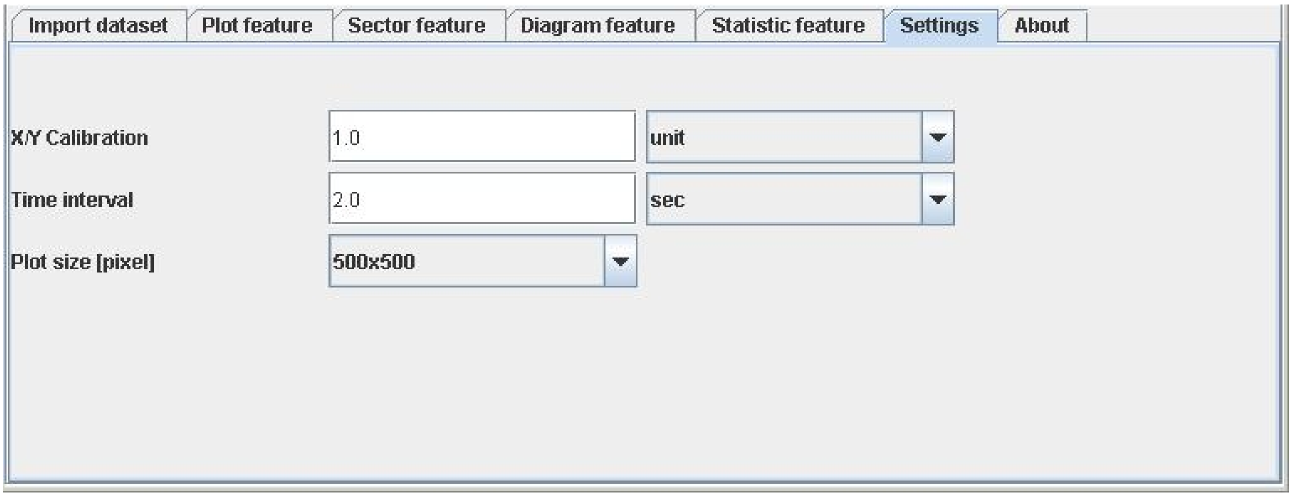
Rayleigh Test

Selected Dataset:	Select a dataset.
Distance from origin:	<u>Use endpoints:</u> Only the endpoints of the trajectories will be used for computation. <u>Endpoints with distance greater than:</u> Only the endpoints of the trajectories with a greater euclidean distance will be used for computation. Demands a threshold value as input. <u>First point with distance greater than:</u> Uses the first point along the trajectory with a greater euclidean distance from the origin. Demands a threshold value as input. See *Horizon Method* **[4]**
Number of used tracks:	Number of used tracks.
p-value:	Computed p-value.

Information about the used Rayleigh test and the statistical hypothesis of the p-value. See **[3]**

Rayleigh Test for vector data

A modification of the Rayleigh test for vector data. See **[5]**



X/Y Calibration: 1.0 unit

Time interval: 2.0 sec

Plot size [pixel]: 500x500

figure 14: Settings

Settings

X/Y Calibration:	Converts pixel to linear measure. Depending on the microscopy and camera parameters.
Time interval:	Adjustment of the time between the discrete records. This value is needed for the computation of the velocities.
Plot size:	Sets the size of the plots.

Definitions

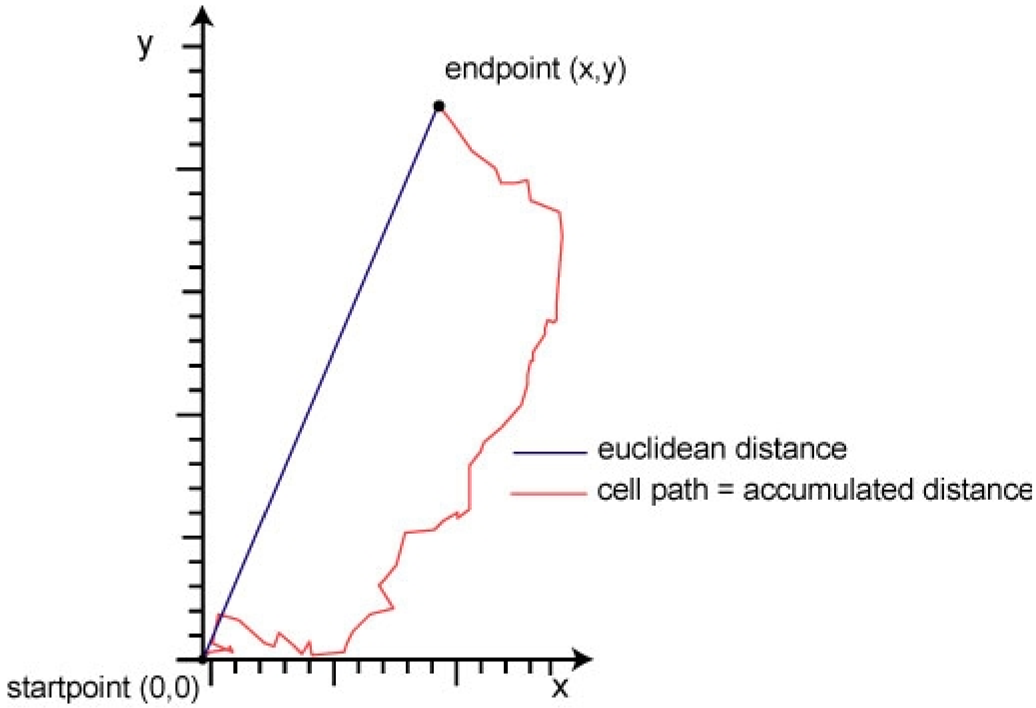


Figure 15: Accumulated and euclidean distance

Directionality: Value for the directionality of motion.

$$Directionality = \frac{euclidean\ distance}{accumulated\ distance}$$

$Directionality \rightarrow 1$: straight motion

$Directionality \rightarrow 0$: non straight motion

Center of mass: Center of mass of all endpoints.

$$x = \frac{1}{n} \sum Endpoint\ x\ value$$

$$y = \frac{1}{n} \sum Endpoint\ y\ value$$

$$Length = \sqrt{x^2 + y^2}$$

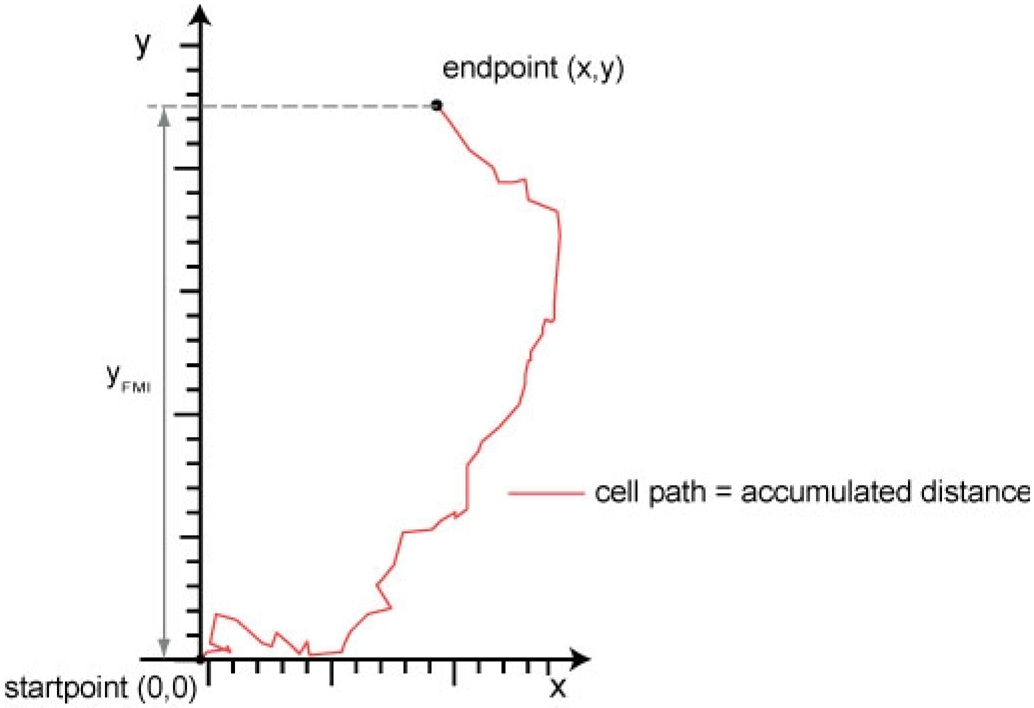


Figure 16: y FMI

FMI (Forward Migration Index): [2]

$$x\,FMI = \frac{x_{FMI}}{\text{accumulated distance}} \qquad y\,FMI = \frac{y_{FMI}}{\text{accumulated distance}}$$

Position angular sector field

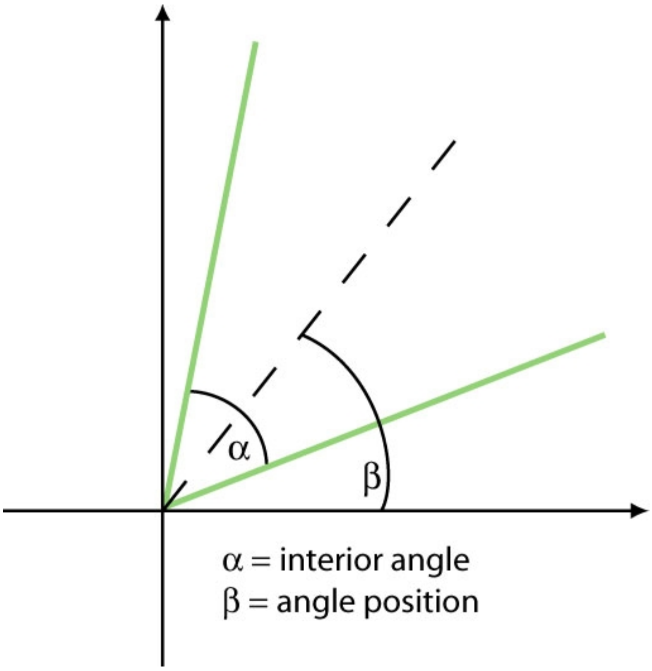
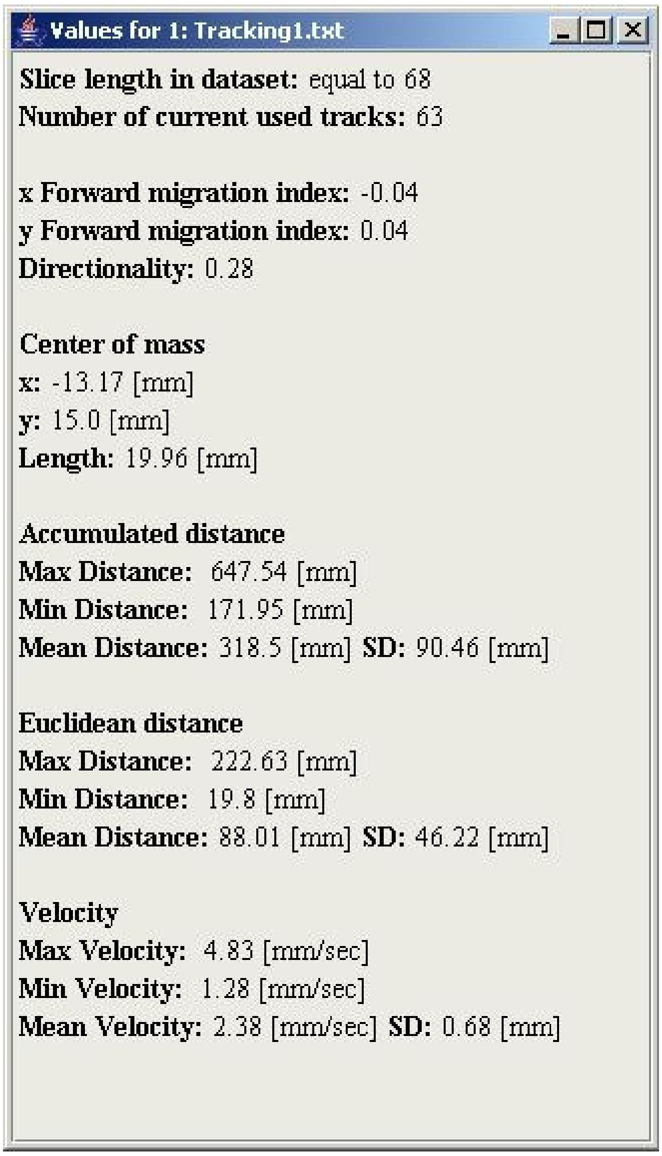


Figure 17: Position angular sector field



Slice length in dataset: equal to 68
Number of current used tracks: 63

x Forward migration index: -0.04
y Forward migration index: 0.04
Directionality: 0.28

Center of mass
x: -13.17 [mm]
y: 15.0 [mm]
Length: 19.96 [mm]

Accumulated distance
Max Distance: 647.54 [mm]
Min Distance: 171.95 [mm]
Mean Distance: 318.5 [mm] **SD:** 90.46 [mm]

Euclidean distance
Max Distance: 222.63 [mm]
Min Distance: 19.8 [mm]
Mean Distance: 88.01 [mm] **SD:** 46.22 [mm]

Velocity
Max Velocity: 4.83 [mm/sec]
Min Velocity: 1.28 [mm/sec]
Mean Velocity: 2.38 [mm/sec] **SD:** 0.68 [mm]

Figure 18: Show info

x/y Forward migration indices **[2]**: Averaged indices over all tracks.

$$x\ Forward\ migration\ index = \frac{1}{n}\sum_{i=1}^{n} x\ FMI_i$$

$$y\ Forward\ migration\ index = \frac{1}{n}\sum_{i=1}^{n} y\ FMI_i$$

Directionality: Averaged index over all tracks.

$$Directionality = \frac{1}{n}\sum_{i=1}^{n} Directionality_i$$

SD: Standard deviation

Installation

1. Copy the *chemotaxis_tool.jar* file in the *ImageJ plugin* folder.
2. Restart *ImageJ*.
3. *Chemotaxis Tool* can now be selected and started inside the *ImageJ plugin* menu.

The current *ImageJ* Version can be downloaded from http://rsb.info.nih.gov/ij/.

If there are problems starting the *Chemotaxis Tool* please download the bundled *ImageJ* Version with Java from the *ImageJ* page.

For questions and suggestions please mail to mailto:gtrapp@ibidi.de

References

[1] Mardia Kanti V., Jupp Peter E., 1999, Directional Statistics, Wiley Series

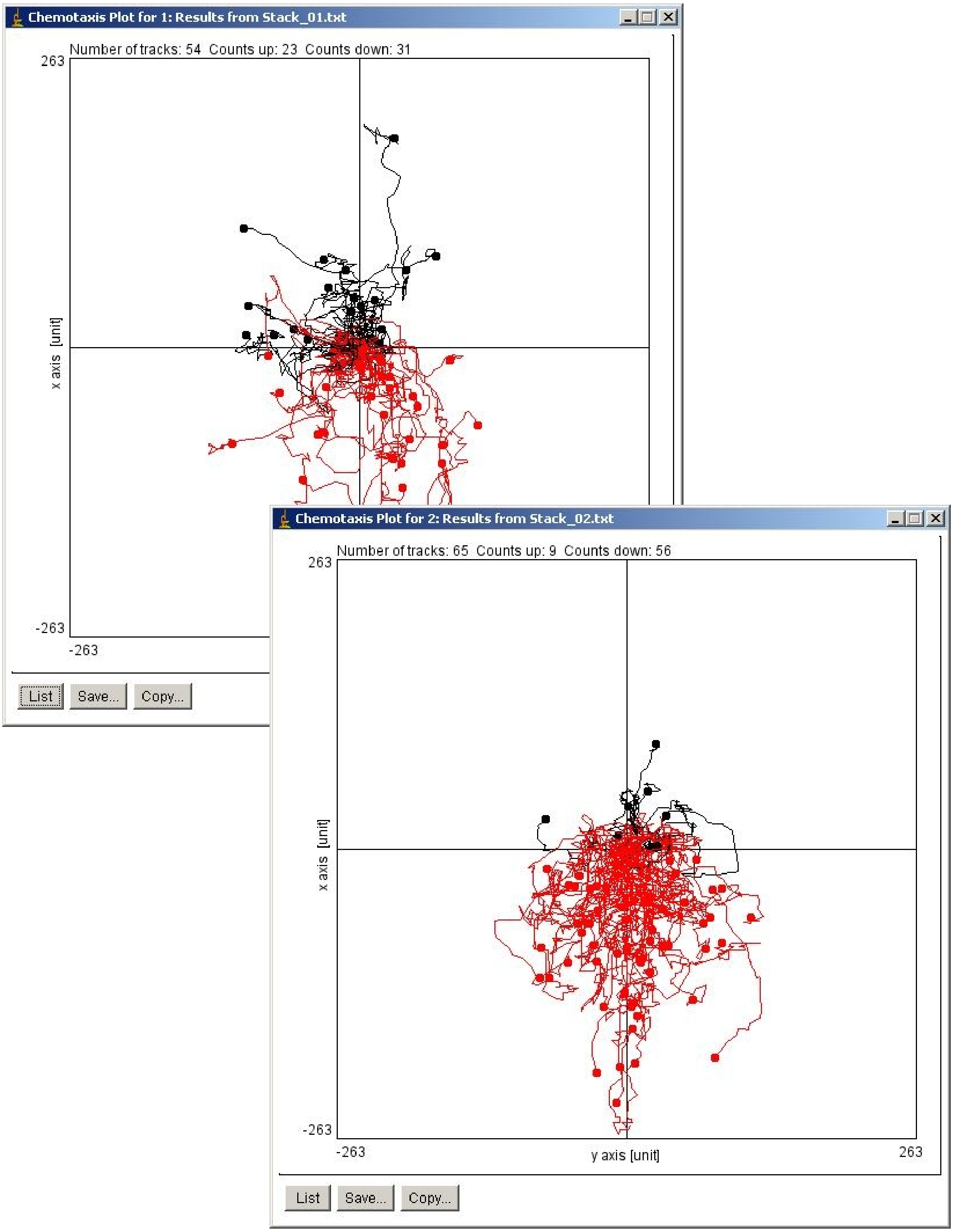
[2] Foxman Ellen F., Kunkel Eric J., Butcher Eugene C., 1999, Integrating Conflicting Chemotactic Signals: The Role of Memory in Leukocyte Navigation, The Journal of Cell Biology, Volume 147, 577-587

[3] N.I. Fisher, 1993, Statistical analysis of circular data

[4] Zicha D., Dunn G., Jones G., 1997, Analyzing Chemotaxis Using the Dunn Direct-Viewing Chamber, Methods in Molecular Biology, Volume 75, 449-457

[5] Moore BR., 1980, A modification of the Rayleigh test for vector data, Biometrika, Volume 67, 175-180

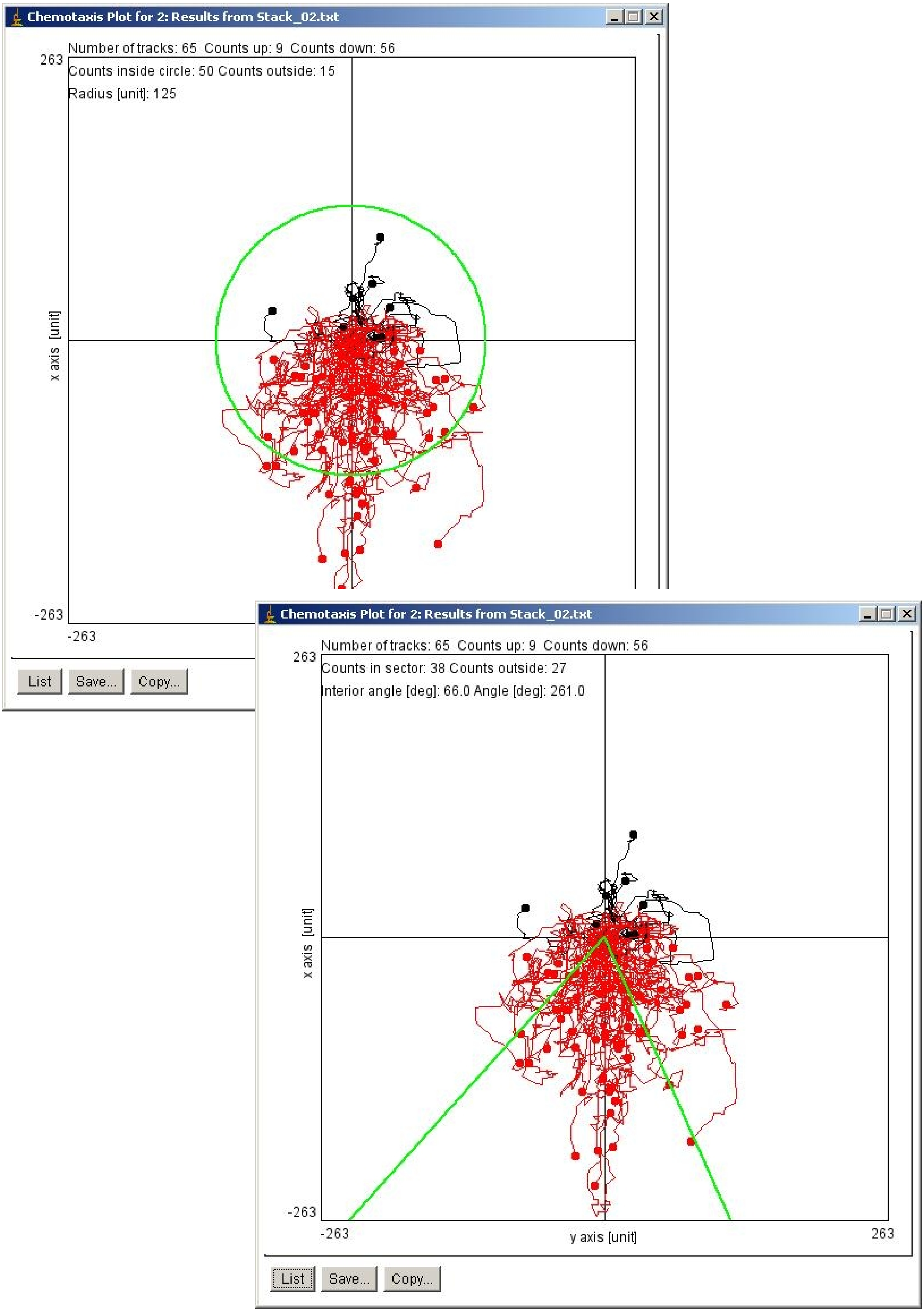
Appendix Diagrams and Plots



Number of tracks: 54 Counts up: 23 Counts down: 31

Number of tracks: 65 Counts up: 9 Counts down: 56

Figure 19: Two plots from different datasets



Chemotaxis Plot for 2: Results from Stack_02.txt

Number of tracks: 65 Counts up: 9 Counts down: 56

Counts inside circle: 50 Counts outside: 15

Radius [unit]: 125

Chemotaxis Plot for 2: Results from Stack_02.txt

Number of tracks: 65 Counts up: 9 Counts down: 56

Counts in sector: 38 Counts outside: 27

Interior angle [deg]: 66.0 Angle [deg]: 261.0

Figure 20: Sector feature

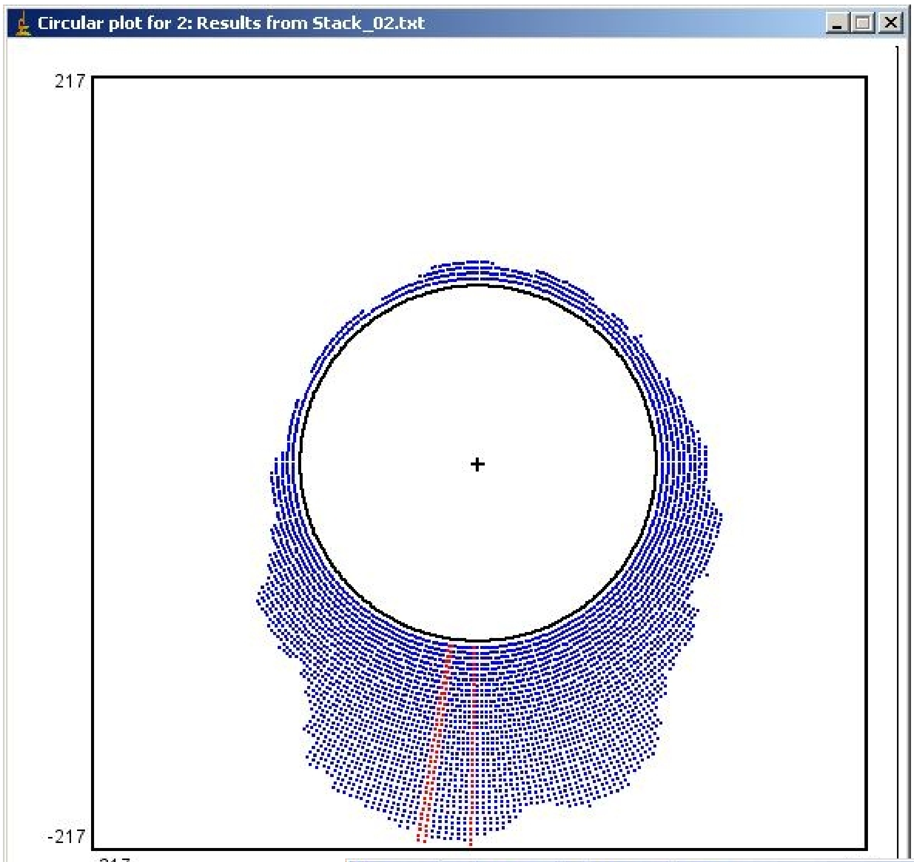


Figure 21: Circular plot

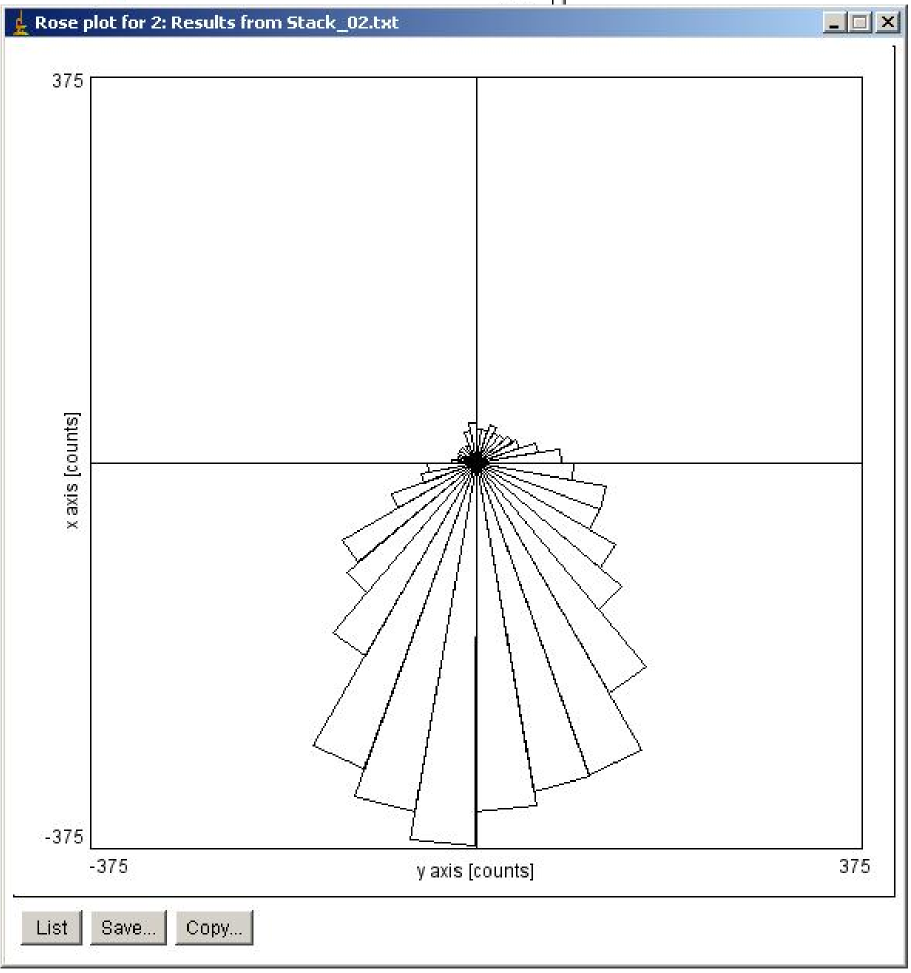


Figure 22: Rose diagram

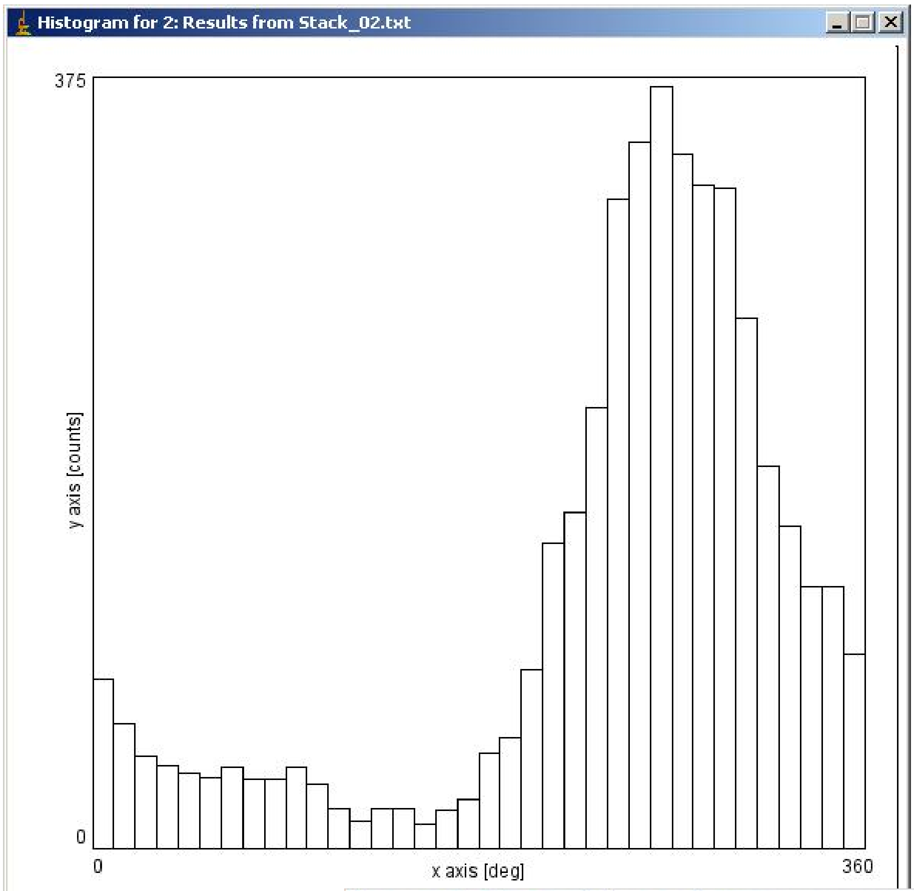


Figure 23: Histogram

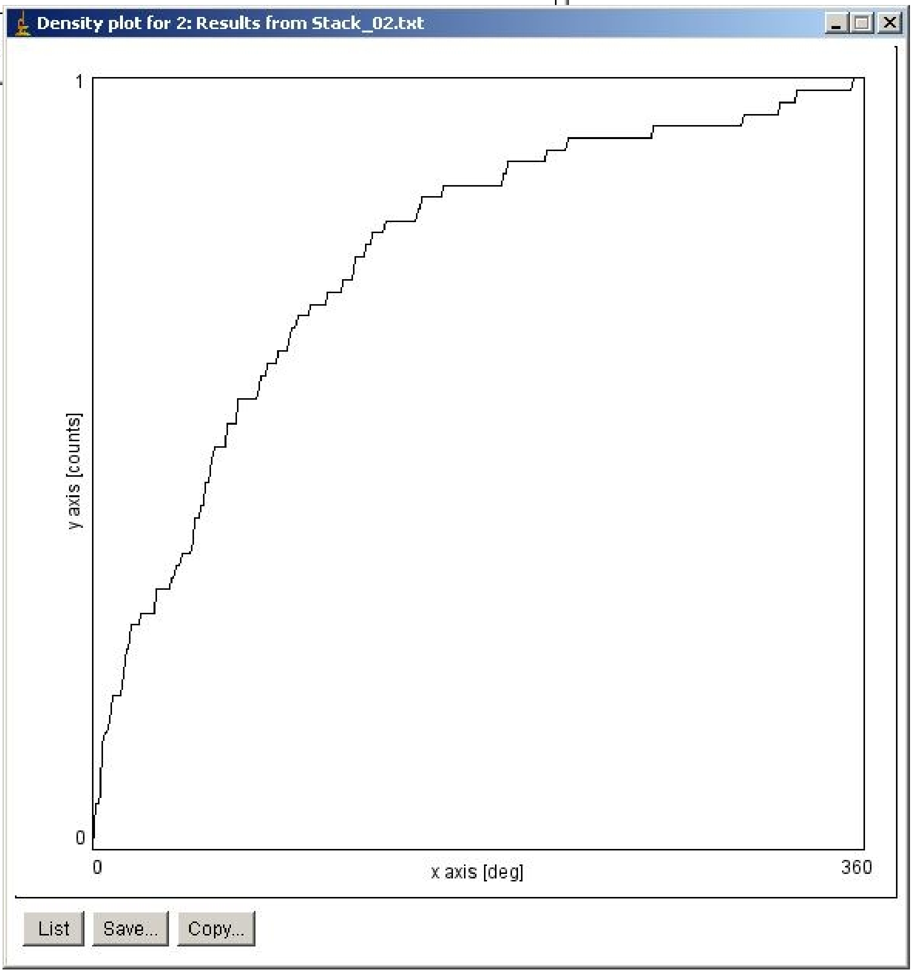


Figure 24: Density plot