

Macro “AG paNoel”

version 1.0.0

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2. Description

This macro was developed under the macro language of [Fiji](#) but it also works on [ImageJ](#) software.

This macro allows to calculate kinetic parameters and represent along the trajectories from x,y coordinates data. The x,y coordinates must be obtained using a third party software employed for cell or particle tracking. For automatic particle tracking we recommend the Fiji/ImageJ plug-in "[Particle Tracker 2D/3D](#)" from the [Mosaic Group](#). And for manual tracking, we recommend the plug-in "[MtrackJ](#)" from [Eric Meijering](#). These two plug-ins have been tested to work with the macro. However, the macro allows to process data obtained from other software which can deliver a trajectory ID, and the "x" and "y" coordinates. Data must be organized in columns as shown in the figure 1.

Datasheet obtained with Particle Tracker

trajectory ID

x,y coordinates

	Trajectory	Frame	x	y	z	m0	m1	m2	m3	m4	NPscore
1	1	0	225.604	89.017	0	1.485	1.343	2.283	4.282	8.738	0.472
2	1	2	223.727	89.983	0	1.326	1.352	2.318	4.407	9.086	0.309
3	1	3	222.029	90.436	0	1.934	1.315	2.233	4.259	8.959	0.256
4	1	4	220.907	91.528	0	1.455	1.321	2.233	4.211	8.688	0.311
5	1	5	219.368	91.823	0	1.080	1.312	2.186	4.030	8.069	0.409
6	1	6	218.435	92.918	0	0.895	1.347	2.288	4.289	8.696	0.298
7	1	7	216.832	93.435	0	1.818	1.332	2.269	4.318	9.000	0.415
8	1	8	215.965	94.279	0	1.524	1.324	2.252	4.286	8.952	0.335
9	1	9	214.336	94.715	0	2.036	1.325	2.224	4.131	8.330	0.381
45	1	44	175.426	118.449	0	1.864	1.311	2.164	3.924	7.666	0.333
46	1	45	174.504	118.924	0	1.681	1.374	2.369	4.516	9.324	0.250
47	1	46	173.417	119.910	0	1.265	1.387	2.416	4.654	9.712	0.250
48	1	47	171.949	120.145	0	1.200	1.344	2.313	4.445	9.350	0.250
49	1	48	171.180	121.273	0	0.980	1.367	2.359	4.504	9.323	0.250
50	1	49	169.791	121.833	0	1.545	1.383	2.409	4.651	9.757	0.250
51	2	0	247.310	144.884	0	1.488	1.334	2.234	4.112	8.154	0.472
52	2	1	247.559	145.174	0	1.813	1.302	2.142	3.876	7.574	0.250
53	2	2	248.796	145.392	0	1.370	1.304	2.155	3.933	7.789	0.309
54	2	3	249.315	145.790	0	1.999	1.287	2.088	3.707	7.073	0.256
55	2	4	249.914	146.072	0	1.356	1.365	2.369	4.578	9.643	0.311
56	2	5	250.885	146.332	0	0.988	1.321	2.211	4.101	8.271	0.409
57	2	6	251.790	146.561	0	0.892	1.288	2.107	3.810	7.484	0.298
58	2	7	252.518	146.920	0	1.734	1.333	2.235	4.125	8.209	0.415

Datasheet obtained with MtrackJ

trajectory ID

x,y coordinates

	Nr	Clust	Track	x [pixel]	y [pixel]	t [sec]	l [val]	Len [pixel]	D2S [pixel]	D2O [pixel]	D2P [pixel]	v [pixel/sec]
1	1	1	1	691.746	85.554	0	144	0.000	0.000	NA	NA	NA
2	2	1	2	695.550	88.053	0	72	4.551	4.551	NA	4.551	NA
3	3	1	3	696.990	94.714	0	58	11.365	10.554	NA	6.814	NA
4	4	1	4	696.181	98.909	0	72	15.638	14.072	NA	4.273	NA
5	5	1	5	695.845	105.541	0	156	22.279	20.403	NA	6.641	NA
6	6	1	6	697.194	110.496	0	100	27.414	25.530	NA	5.135	NA
7	7	1	7	695.669	115.590	0	47	32.731	30.291	NA	5.317	NA
8	8	1	8	696.082	121.341	0	144	38.497	36.048	NA	5.766	NA
9	9	1	9	696.478	124.233	0	47	41.416	38.967	NA	2.919	NA
10	10	1	10	701.472	125.782	0	148	46.645	41.386	NA	5.229	NA
25	25	1	25	703.522	203.905	0	128	143.583	118.935	NA	7.317	NA
26	26	1	26	708.368	207.170	0	169	149.426	122.746	NA	5.843	NA
27	27	1	27	704.226	214.979	0	187	158.265	130.025	NA	8.839	NA
28	28	1	28	706.829	218.475	0	84	162.624	133.774	NA	4.359	NA
29	29	1	29	703.232	222.372	0	169	167.927	137.299	NA	5.303	NA
30	30	2	1	754.525	270.899	0	58	0.000	0.000	NA	NA	NA
31	31	2	2	749.801	268.429	0	119	5.331	5.331	NA	5.331	NA
32	32	2	3	747.074	270.128	0	100	8.544	7.490	NA	3.213	NA
33	33	2	4	741.525	267.188	0	44	14.824	13.519	NA	6.280	NA
34	34	2	5	739.285	262.373	0	62	20.134	17.463	NA	5.310	NA
35	35	2	6	732.965	260.980	0	47	26.606	23.732	NA	6.472	NA
36	36	2	7	732.896	252.633	0	47	34.953	28.310	NA	8.347	NA

Figure 1: Datasheets obtained with Particle Tracker or MtrackJ plug-ins.

The macro allows to calculate different kinetics parameters and represent it along the trajectory with a color code allowing a visual analysis of them. In this version, the macro allows to represent the following parameters:

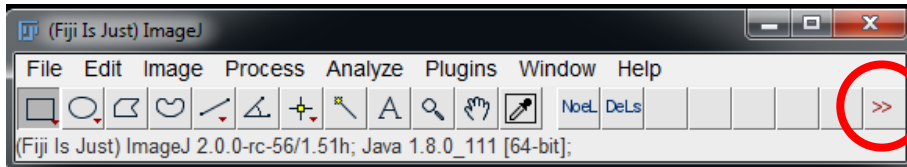
- Dots:** recreate the trajectory by representing the position of the x,y coordinates with dots.
- Lines:** recreate the trajectory joining the x,y coordinates with lines.
- VCL:** calculates the curvilinear velocity and represent it along the trajectory.
- VAP:** calculates the mean velocity and represent it along the trajectory.
- VSL:** calculates the straight-line velocity and represent it along the trajectory.
- Delta VCL:** calculates changes in the VCL between successive pair of x,y coordinates represent it along the trajectory.
- Orientation:** calculates the orientation of the movement and represent if along the trajectory.
- Linearity:** calculates the linearity parameter of the movement and represent if along the trajectory.
- Fractal Dimension:** calculates the fractal dimension of the movement and represent if along the trajectory.
- Intensity:** represents the intensity of fluorescence along the trajectory.

3. Downloading and installing the macro

- Download the macro.
- Save the macro into the folder "...\\Fiji.app\\macros\\toolsets" or "...\\ImageJ\\macros\\toolsets"

4. Opening the macro

1. Open Fiji/ImageJ.
2. Click in the button "More Tools" menu.
3. Select "AG paNoel x.x.x"



"More Tools" menu

5. Running the macro

1. Open the datasheet with the x,y coordinates to analyze (*File > Import > Results...*).
2. Clicking with the mouse **left** button over "Noel" button executes the macro.
3. Clicking with the mouse **right** button over "Noel" button shows a "Dialog Menu" with the *Configuration Options*.

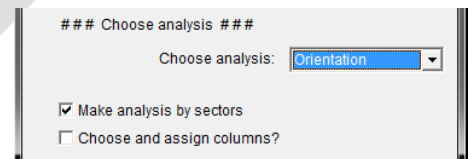
6. Configuration Options

Configuration Options allows to choose the type of analysis and set different options to properly calculate the parameters and represent them.

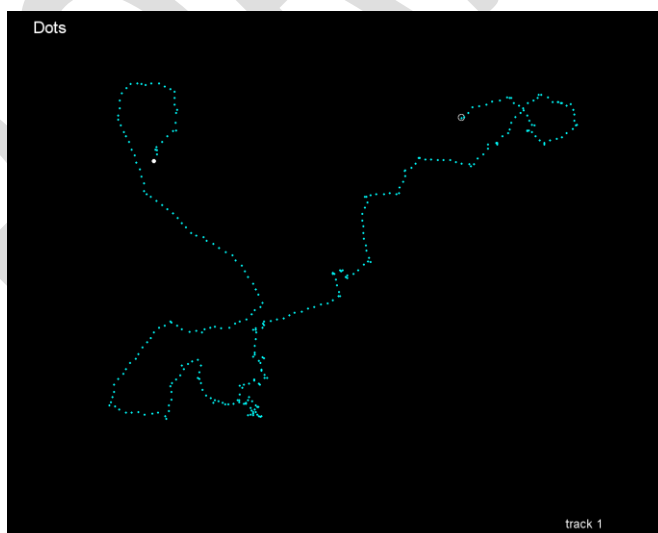
a) ### Choose analysis

i) Choose analysis:

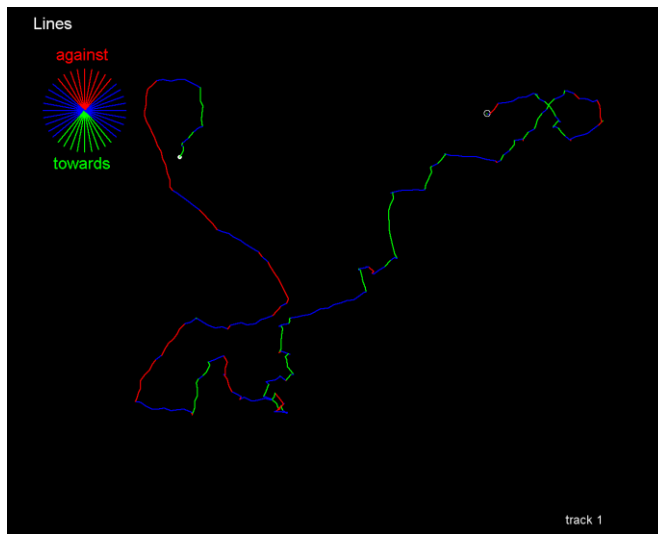
Specifies the type of analysis that will perform



- (1) **Dots**. Recreates the trajectory by representing the position of the x,y coordinates with dots.



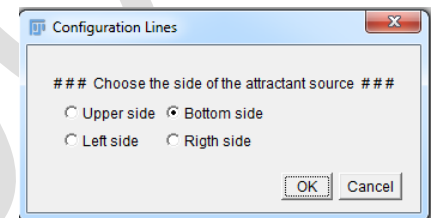
- (2) **Lines**. Recreate the trajectory joining the x,y coordinates with lines.



(a) Configuration Lines

Choose the side of the attractant source

This represents the lines with a color code (see Orientation). This is useful in orientation experiments. If you just want to represent the trajectory the "Dots" representation is recommended.

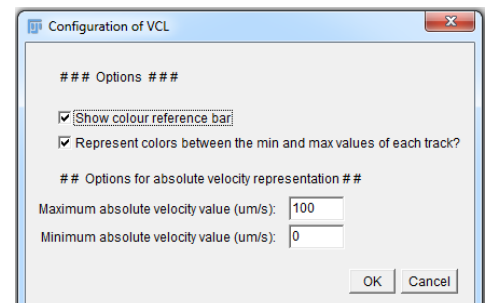


- (3) **VCL**. Calculates the curvilinear velocity and represents it with a color code along the trajectory.

(a) Configuration of VCL

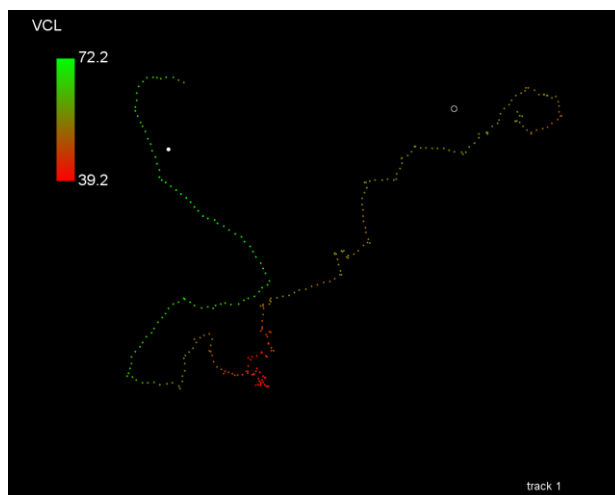
(i) ### Options

1. **Show color reference bar.** If this option is selected, shows the color code bar in the image.
2. **Represent colors between the min and the max values of each track?** If this option is selected, the color code range of the reference bar is set between the minimum and the maximum value of velocity of each trajectory. If it is not selected, the color code range of the reference bar is set for all the trajectories between the minimum and the maximum values selected by the user in *### Options for absolute velocity representation ###*.



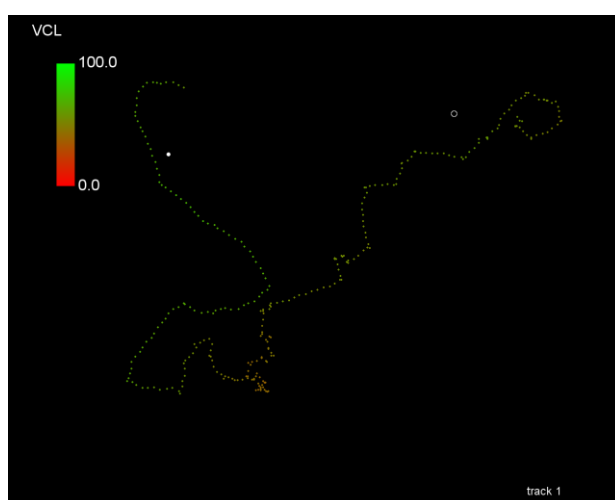
(ii) ### Options for absolute velocity representation

1. **Maximum absolute velocity value ($\mu\text{m/s}$).** Sets the maximum value of the color code range in the reference bar.
2. **Minimum absolute velocity value ($\mu\text{m/s}$).** Sets the minimum value of the color code range in the reference bar.



Representation of relative VCL. The min and the max value in the color code scale bar is set on each trajectory min and max VCL value.

(when "Represent colors between the min and the max values of each track?" IS selected)

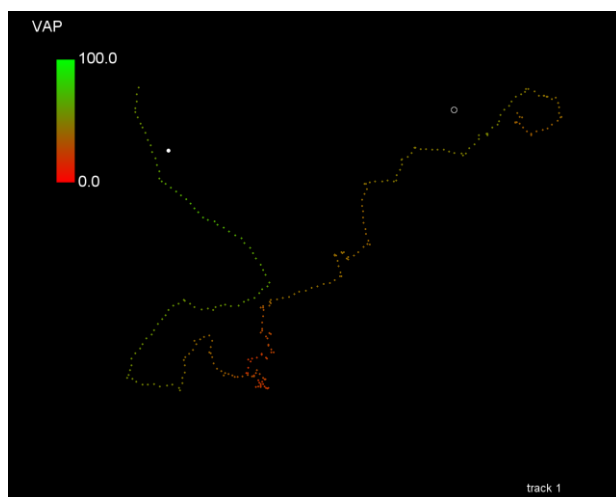


Representation of absolute VCL. The min and the max value in the color code scale bar is set by the user (between 0 and 100 $\mu\text{m/s}$ in this example) and applies for all trajectories.

(when "Represent colors between the min and the max values of each track?" IS NOT selected)

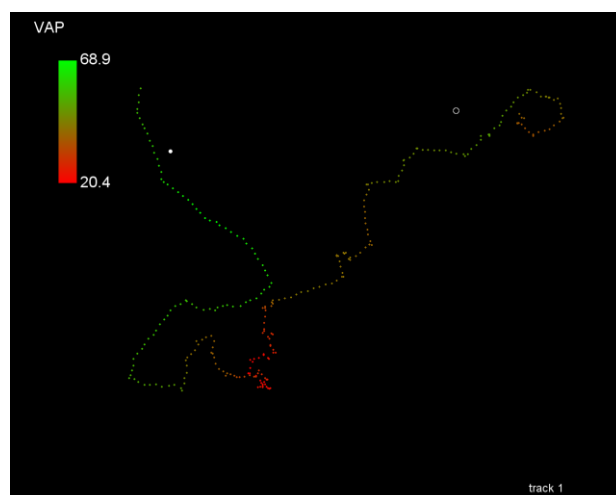
- (4) **VAP and VAPi.** Calculates the mean velocity as defined by Mortimer et al. (1995) and represent it with a color code along the trajectory. To calculate the VAP, first the mean x,y coordinates are determined by averaging 10 points besides the actual (5 before and 5 after). Then, the VAP is calculated as the VCL of the mean x,y coordinates. VAP calculation is recommended for videos recorded at least at 30Hz.

VAPi is a modification of the VAP for videos recorded between 5-30 Hz. The mean x,y coordinates is calculated by averaging one point before and one point after besides the current point. Then the VAP is calculated as the VCL of the mean x,y coordinates



Representation of relative VAP. The min and the max value in the color code scale bar is set on each trajectory min and max VAP value.

(when "Represent colors between the min and the max values of each track?" IS selected)

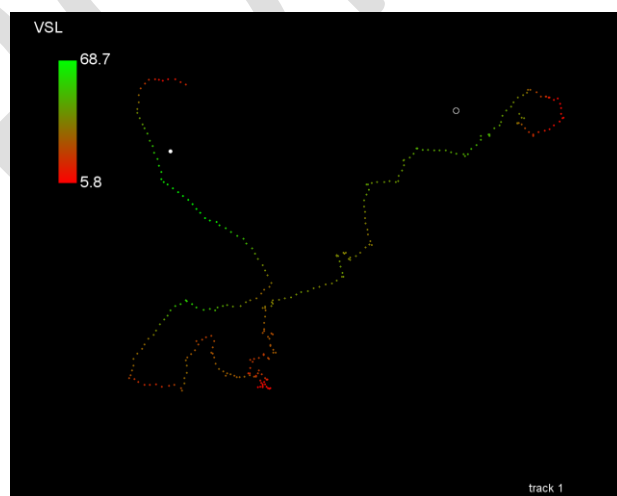


Representation of absolute VAP. The min and the max value in the color code scale bar is set by the user (between 0 and 100 $\mu\text{m/s}$ in this example) and applies for all trajectories.

(when "Represent colors between the min and the max values of each track?" IS NOT selected)

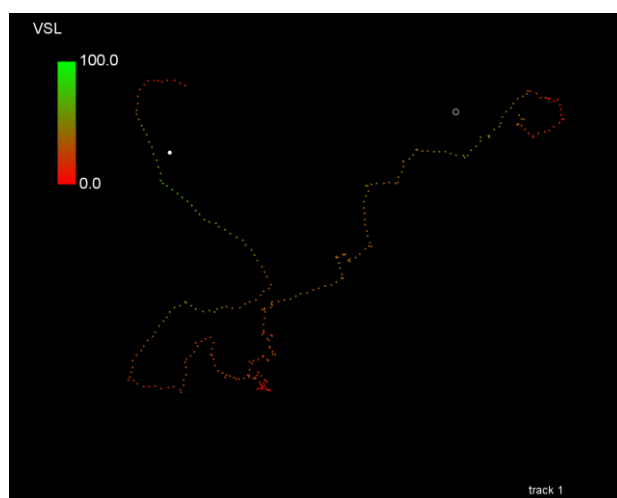
(a) Configuration of VAP or VAPi. (See Configuration of VCL)

(5) **VSL.** Calculates the straight-line velocity and represent it with a color code along the trajectory.



Representation of relative VSL. The min and the max value in the color code scale bar is set on each trajectory min and max VSL value.

(when "Represent colors between the min and the max values of each track?" IS selected)

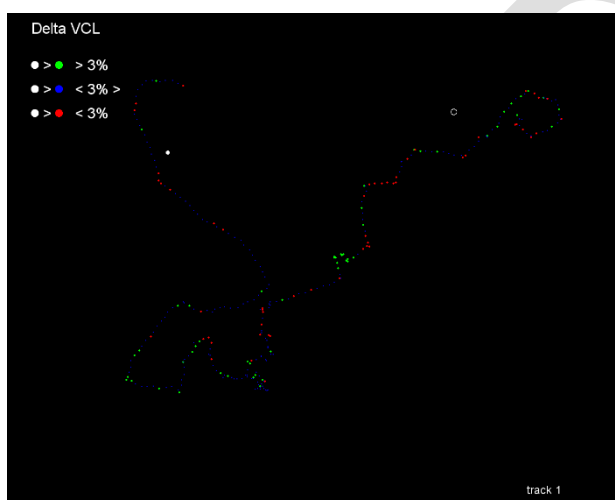


(a) Configuration of VSL. (See Configuration of VCL)

Representation of absolute VSL. The min and the max value in the color code scale bar is set by the user (between 0 and 100 $\mu\text{m/s}$ in this example) and applies for all trajectories.

(when "Represent colors between the min and the max values of each track?" IS NOT selected)

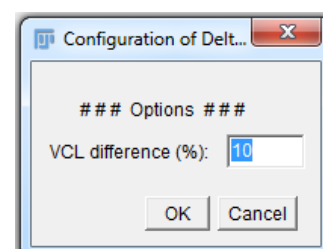
- (6) **Delta VCL.** Represents the changes in the VCL which are higher than threshold between two successive pair of x,y coordinates and are represented as color lines along the trajectory.



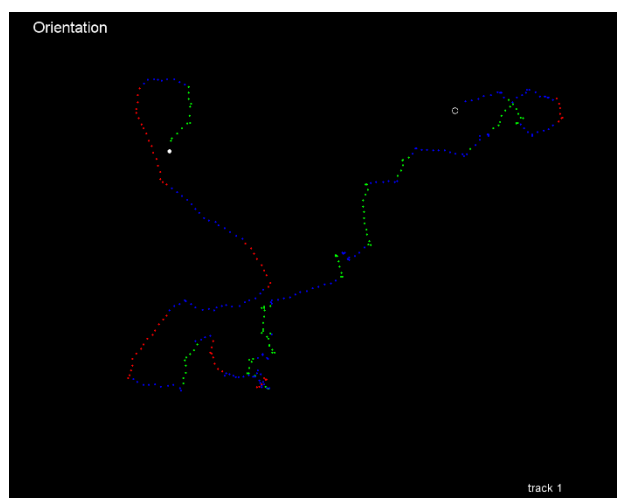
(a) Configuration of Delta VCL

(i) ### Options ###

- VCL difference (%).** Set the threshold value of detection for changes in velocity between two consecutives values. It means if the difference between two consecutiva points are higher than the threshold they are represented in color.



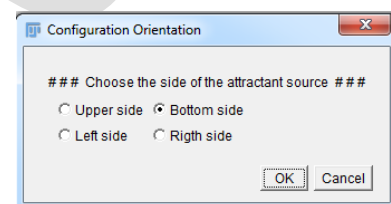
- (7) **Orientation.** Calculates the orientation of the movement and represent it with color dots along the trajectory. The color of the dots represents the direction of the movement. (**Green**, moves toward the gradient of the substance source; **Blue**, moves perpendicular to the gradient of the substance source or **Red**, moves against the gradient of the substance source).



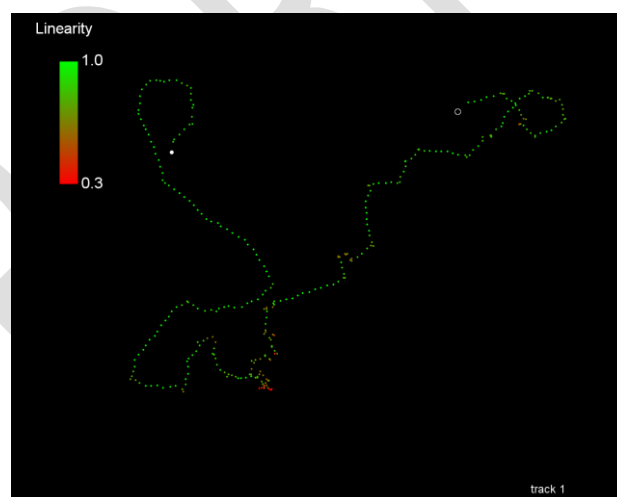
(a) Configuration Orientation

(i) ### Choose the side of the attractant source ###

Set the side of the image where the test substance was placed. In this version, the orientation can be set only in linear gradients matching with one of the side of the image.



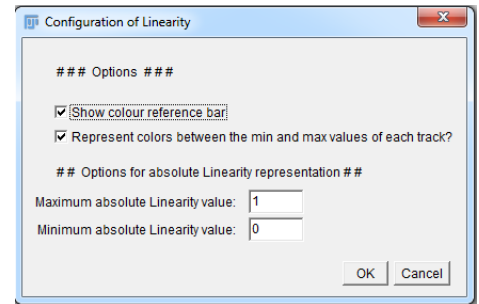
- (8) **Linearity** - calculates the linearity parameter of the movement and represent if with color dots along the trajectory.



(a) Configuration of Linearity

(i) ### Options ###

1. **Show color reference bar.** If this option is selected, shows the color code bar in the image.
2. **Represent colors between the min and the max values of each track?** If this option is selected, the color code range of the reference bar is set between the minimum and the maximum value of Linearity of each trajectory. If it is no selected, the color code range of the reference bar is set for all the trajectories between the minimum and the maximum values selected by the user in **### Options for absolute Linearity representation ###**.



(ii) **### Options for absolute Linearity representation ###**.

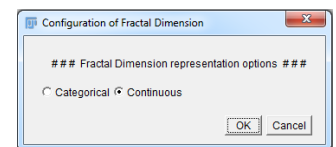
1. **Maximum absolute Linearity value.** Sets the maximum value of the color code range in the reference bar.
2. **Minimum absolute Linearity value.** Sets the minimum value of the color code range in the reference bar.

(9) **Fractal Dimension** - calculates the fractal dimension of the movement and represent it as color dots along the trajectory.

(a) **Configuration of Fractal Dimension**

(i) **### Fractal Dimension representation options ###**

1. **Categorical.** If this option is selected, shows the fractal dimension as a preset "type of movement". See figure caption.
2. **Continuous.** If this option is selected, represent the fractal dimension value as a color dot.

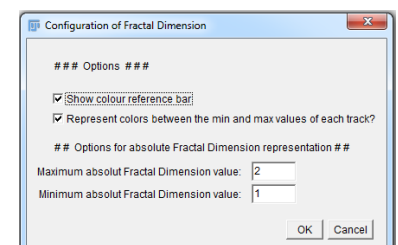


a. **Configuration of Fractal Dimension**

Options

Show color reference bar. If this option is selected, shows the color code bar in the image.

Represent colors between the min and the max values of each track? If this option is selected, the color code range of the reference bar is set between the minimum and the maximum value of Fractal Dimension of each trajectory. If it is no selected, the color code range of the reference bar is set for all the trajectories between the minimum and the maximum

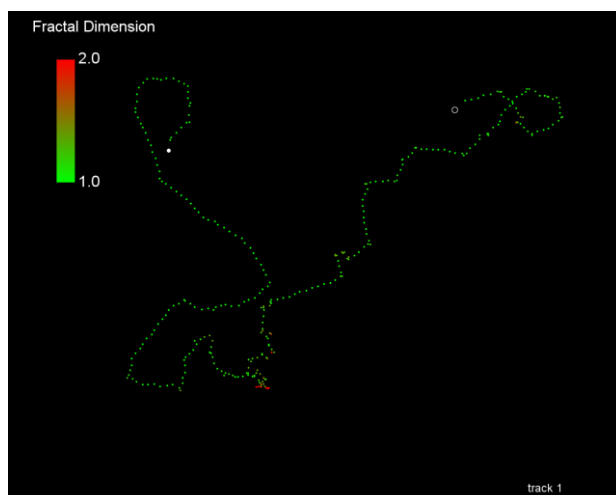


values selected by the user in ### Options for absolute Fractal Dimension representation ###.

Options for absolute Fractal Dimension representation ###.

Maximum absolute Fractal Dimension value. Sets the maximum value of the color code range in the reference bar.

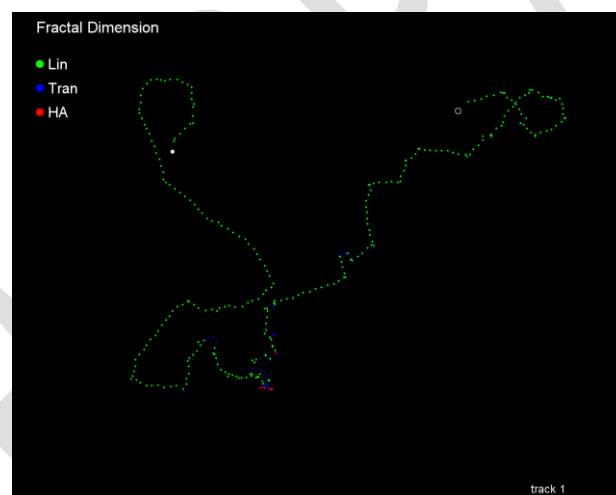
Minimum absolute Fractal Dimension value. Sets the minimum value of the color code range in the reference bar.



Quantitative representation of the absolute value of Fractal Dimension. The min and the max value in the color code scale bar is set by the user (between 1 and 2 in this example) and applies for all trajectories.

(when "Represent colors between the min and the max values of each track?" IS NOT selected)

A **Quantitative representation of the relative value of Fractal Dimension** can be represented when the option "Represent colors between the min and the max values of each track?" IS NOT selected.



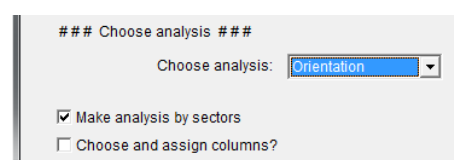
Qualitative representation of the Fractal Dimension. The color of the dots represents the type of movement. Green, indicates linear type of movement; Blue, indicates transitional type of movement and Red, indicates hyperactivated type of movement.

(FD: $1 < \text{Lineal} < 1.3 < \text{Transitional} < 1.8 < \text{Hyperactivated} < 2$)

(10) **Intensity:** represents the intensity of fluorescence along the trajectory.

ii) Make analysis by sectors:

Allows to make analysis of the tracks in determined regions of the image

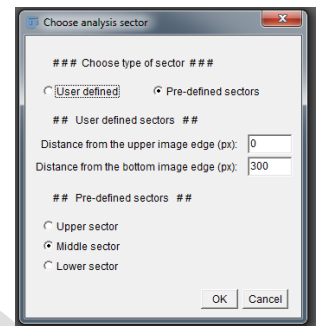


Choose analysis sector options.

Choose analysis sector

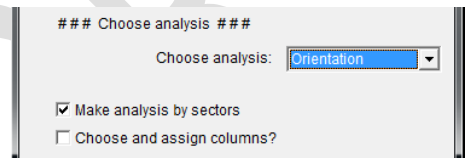
Note: the trajectories drawn starts in the sector but can end in other sectors. This is particularly useful when analyzing orientation to avoid trajectories from the borders.

- (1) **User defined:** Defines the area counting the pixels from the bottom of the image. Maximum distance is limited by the height of the image.
- (2) **Pre-defined sectors:** split the image area in 3 sectors and establishes in which area is performed the analysis.



iii) Choose and assign columns?

This allows to define the name of the columns for the Track ID, x, y, and intensity values. The name of the columns is pre-defined, in case the macro doesn't find the pre-determined names it creates a dialog box so the user can define the variables. Needs a result table open.



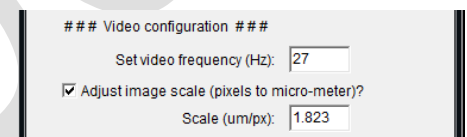
b) ### Video Configuration

i) Set video frequency (Hz)

- (1) Establishes the frequency of the video.

ii) Adjust image scale (pixels to micro-meter)?

- (1) Calculate the Velocity parameters in metric system (micro-meter/second).



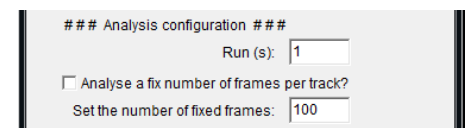
c) ### Analysis configuration

i) Run (s)

- (1) Establishes the length of the trajectory from which the parameters are estimated. By default is set to 1 sec (the number of points included will depend on the frequency of the video).

ii) Analyse a fix number of frames per track?

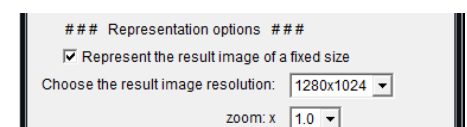
- (1) If checked, a defined number of points per track will be analysed. If the tracks have less number of points they will not be analysed. If the track has more number of points, the exceeding points will not be analysed.



d) ### Representation options

i) Represent the result image of a fixed size

- (1) If it is checked, will draw all the trajectories in a predefined image resolution. Set by "Choose the result image resolution"



- (2) If is unchecked, will fit the image resolution to the size of the trajectory width and high. This may requires an offset correction, if the track is not centered.

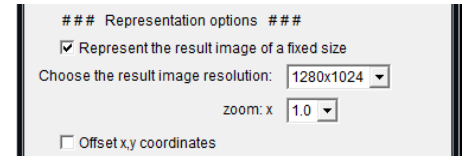
ii) **Zoom**

- (1) Amplifies the images resolution to obtain better points separation in complex trajectories. Only modifies the representation resolution, not the parameter values.

e) ### Representation options ###

i) **Represent the result image of a fixed size**

- (1) If it is checked, will draw all the trajectories in a predefined image resolution. Set by "Choose the result image resolution"
- (2) If is unchecked, will fit the image resolution to the size of the trajectory width and high. This may require an offset correction, if the track is not centered.



ii) **Zoom: x**

- (1) Amplifies the images resolution to obtain better points separation in complex trajectories. Only modifies the representation resolution, not the parameter values.

iii) **Offset x,y coordinates**

- (1) Modifies the position of the track in the imagej. This is useful when working with a standalone trajectory.

f) ADVANCED. Change performance optimization

i) **Represent the result image of a fixed size**

- (1) If it is checked, will draw all the trajectories in a predefined image resolution. Set by "Choose the result image resolution"
- (2) If is unchecked, will fit the image resolution to the size of the trajectory width and high. This may require an offset correction, if the track is not centered.

