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Calculating track parameters from data obtained with Syntech servo-spheres using TRACKanalysis.xls

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An insect walking on the servo-sphere generates an enormous amount of data. Every tenth of a second the XY coordinates are stored, so in just five minutes of walking 3000 X- and 3000 Y-data points are generated. The operator of the servo-sphere is, thus, confronted with a difficult task. In order to analyze these data sets, I developed a set of calculations in Microsoft Excel, TRACKanalysis.xls, based on my experience with servo-sphere tracks and insect orientation. The set of calculations are meant to reduce the data to those parameters which describe the characteristics of final walking tracks and, in addition, contain relevant information about the dynamics of walking behavior.

Calculating track parameters

While additional parameters may be applied, based on my experience, those chosen here best describe the behavior of the insect and are easy to calculate.

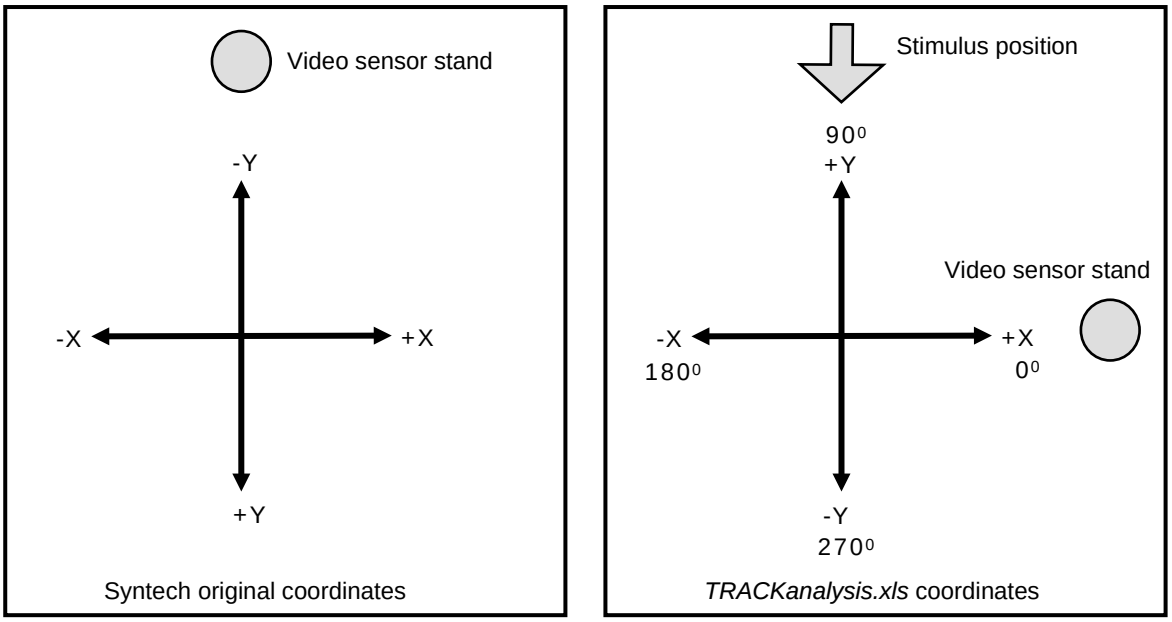
- **Walking speed** is track length per s in mm/s, graphically shown in time.
- **Average speed** is average of walking speeds (including zero speeds) in mm/s.
- **Standard deviation of speed** is standard deviation of average speed (n-1 method) in mm/s.
- **Track length** is the total length of the walking track (sum of track lengths per s) in mm.
- **Vector length** is the net displacement from the origin, in mm.
- **Straightness** is the quotient of the vector length and the track length, ranges from 0 to 1.
- **Sine of the vector angle**, ranges from -1 to +1.
- **Upward length** is the net upward displacement (movement toward stimulus), in mm.
- **Upward straightness** is the quotient of upward length and the track length, ranges from -1 to +1.

The parameters need some further comments. **Walking speed** and **track length** describe more or less the same, although the graphical presentation of walking speeds in time is more dynamic than the final track lengths after periods of time. The **straightness** describes how straight or tortuous is the track of the insect. In general, the more tortuous the track the lower the walking speed will be as insects slow down when turning. **Vector length** illustrates the net displacement from the release point. The **sine of the vector angle** shows preference for a particular angle, and should be interpreted in combination with the vector length. Using the sine of the vector angle is more appropriate than just the vector angle as degrees are circularly distributed, and, therefore, need special statistics. Degrees may even give false numerical impressions, *e.g.*, the distance between 10^0 and 360^0 is smaller than between 300^0 and 360^0. Experiments on the servo-sphere are frequently designed to study the responses to a stimulus coming from a particular direction (the classical taxes), although kinetic responses can be studied as well. **Upward length** and **upward straightness** are selected when insects are supposed to move toward or away from the stimulus source. Upward length is the displacement toward the source of stimulation, a negative sign indicates that the insect moves away from the source. The parameter upward straightness describes how straight the insect is moving towards or away from the source. This parameter is very powerful in discrimination between control and stimulus situations.

Instructions for TRACKanalysis.xls

Background information

Data storage is by Syntech servo-spheres as *csv* files. These files can be imported into Microsoft Excel: columns A and B are reserved for the markers, columns C and D are, respectively, the X and Y coordinates in counts of pulses coming from pulse generators recording the compensating movement of the servo-sphere. Cell E1 is reserved for comments. Each row in *xls* files represents one tenth of a second. Coordinates of the Syntech servo-sphere are set at the factory as shown in the illustration below left. With the small servo-sphere the video sensor stand represents the microscope stand. However, it is more convenient to use the coordinate system shown below at the right. TRACKanalysis.xls makes the appropriate changes for working with the coordinate system on the right, by reading column D as X pulses and column C as Y pulses and changing the sign of both. Thus, tracks recorded in *csv* files are rotated 90^0 to the right.



In TRACKanalysis.xls, walking speeds are calculated per second and expressed in mm, where 10 pulses are 1 mm. The parameters are calculated for the whole period of 5 minutes, and, in addition, for 5 successive periods of 1 minute. The first second of the recording period is not used, so **one should record for at least 301 seconds** to obtain proper means for the period of 5 minutes. Walking speeds are graphically shown during 5 minutes. The graph of the walking track can be rescaled manually by making use of the Excel features: right mouse click on axis, format axis and select scale.

Importing data in TRACKanalysis.xls

Open TRACKanalysis.xls, open the *csv* file, select columns A-E in the *csv* file and copy, then select cell A1 in TRACKanalysis.xls, and paste. Look at print preview; when satisfied print. When dissatisfied with output, rescale axes of track plot (see above), or adjust print area, paper size and/or margins. **Do not store files.**

Advice for recording walking tracks

For the proper interpretation of orientation behavior, one likes to study the responses of insects to one stimulus at the time and to avoid effects coming from unknown or unforeseen sources. Apart from the stimulus source under study, the **direct surroundings** of the servo-sphere should be as stimuli free as possible. Black or white curtains, or cylinders made of paper or metal around the servo-sphere will help to set the proper conditions. **Light intensity** should be regulated to levels at which the insect under study is calm and does not show escape behavior. Ordinary light bulbs may be used as normal fluorescent lamps (100/120 Hz flicker) may have undesirable effects on the insect movements. One should avoid any **positional bias** by, *e.g.*, an uneven distribution of light intensities. **Preconditioning** of insects is essential: insects should be starved before moving towards food odors, mated females may not respond to sex pheromones, or prior experience with food, followed by starvation, increases insect responses towards food odors. Always observe visually whether the insect is in **good shape**, does not miss legs or walks to one side. Under **control conditions**, *i.e.*, without stimulation, insects should walk in circles, moving to one side and then sharply turning to make circles in the opposite direction. The size of these circles is related to size of the insect and its walking speed (Visser, unpubl.). When stimulation from the surroundings is increased, walking is straighter but may still have small loops. Adapt the settings of the servo-sphere (gain and filter) so that the insect can somewhat displace from the center but not to a degree that **gravitational forces** on the slope of the sphere provides a cue for orientation. One should be careful that the insect stays really on top of the sphere and fix the camera accordingly. The experiments on a servo-sphere differ from normal walking of an insect as **visual feedback** from the surroundings is lacking ("open loop"). Last but not least, make the proper experimental design so that data can be analyzed **statistically** and do not trust your own capabilities just looking at walking tracks.

Selected literature

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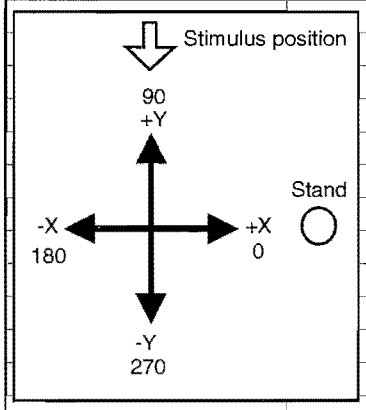
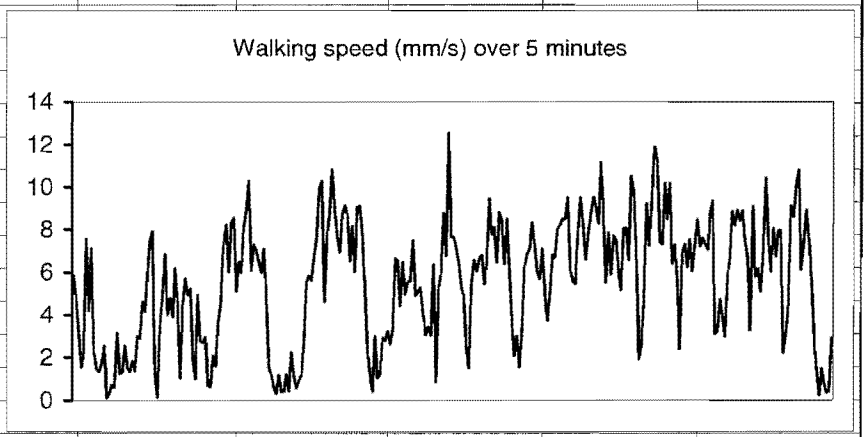
Time period	all 5 minutes	1st minute	2nd minute	3rd minute	4th minute	5th minute
Average speed (mm/s)	5.54	3.27	5.49	5.39	7.39	6.17
Sd Speed (mm/s)	2.87	2.13	3.25	2.36	2.11	2.73
Track length (mm)	1662	196	329	323	443	370
Vector length (mm)	779	134	105	245	388	334
Straightness	0.469	0.686	0.319	0.758	0.876	0.902
Sine of vector angle	-0.054	0.729	0.699	0.587	-0.282	-0.742
Upward length (mm)	-42	98	73	144	-110	-248
Upward straightness	-0.025	0.500	0.223	0.445	-0.247	-0.670





file CPB1.csv: CPB female

TOTAL PERIOD HAS BEEN (in s): 300