TrackSphere
LOCOMOTION COMPENSATOR
Model LC-300

User guide

SYNTECH

INTRODUCTION

The Syntech TrackSphere LC-300 is functionally similar to the locomotion compensator for insects first constructed and described by Kramer (1976).
It consists of a sphere, on top of which the insect is placed, and which is rotated opposite to the insect's displacements by means of two electric motors. The motors are driven by electrical commands proportional to the displacement of the insect measured by an optical detector located above the insect. Two encoders orthogonally contacting the sphere transmit the rotational movements to a computer, where the displacements are stored as incremental X and Y coordinates.
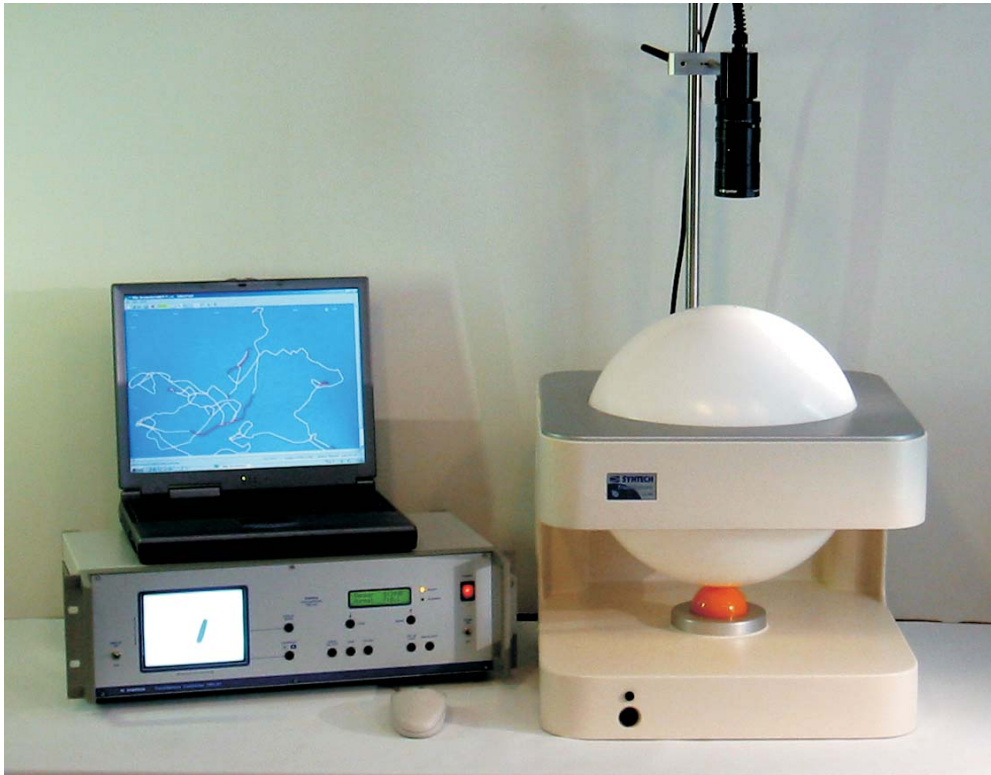
The instrument can be used for a wide range of orientation experiments (Kramer, 1976, Weber et al.,1981;Tobin and Bell,1986) and allows manipulation of the insect's environment over long periods and in an unlimited virtual area. Although the method is straightforward and very attractive as a research tool it is not widely applied because of the complicated construction, difficult operation, and high cost. Moreover, there is a risk for contamination of the sphere surface when using high concentrations and sensitive insects, and the insect needs to be applied with a tiny reflector on its back for the displacement sensor to work properly.

The design of the Syntech LC-300 aims to overcome several of the weaknesses of conventional servospheres:
* The size of the sphere is limited to 300 mm resulting in a light and compact construction.
* The sphere is supported by an aerostatic spherical bearing, which is noiseless and easy to clean.
* The displacement detector is based on a CMOS video sensor followed by a digital signal processor eliminating the need for a reflector on the insect.
* Only two parameters in the feedback loop between detector and motor drives need to be adjusted by the operator: 1. feedback gain , and 2. Time Constant of the feedback response.
* A real-time video display facilitates focusing the displacement sensor and proper adjustment of the illumination (Infrared and visible).

Despite these improvements successful operation of the LC-300 requires the operator to be well familiar with the principle of operation, the various adjustments to be made, and the experimental limitations of the system.
It is recommended to 'play' with the instrument until sufficient experience has been gained to perform explorative experiments.
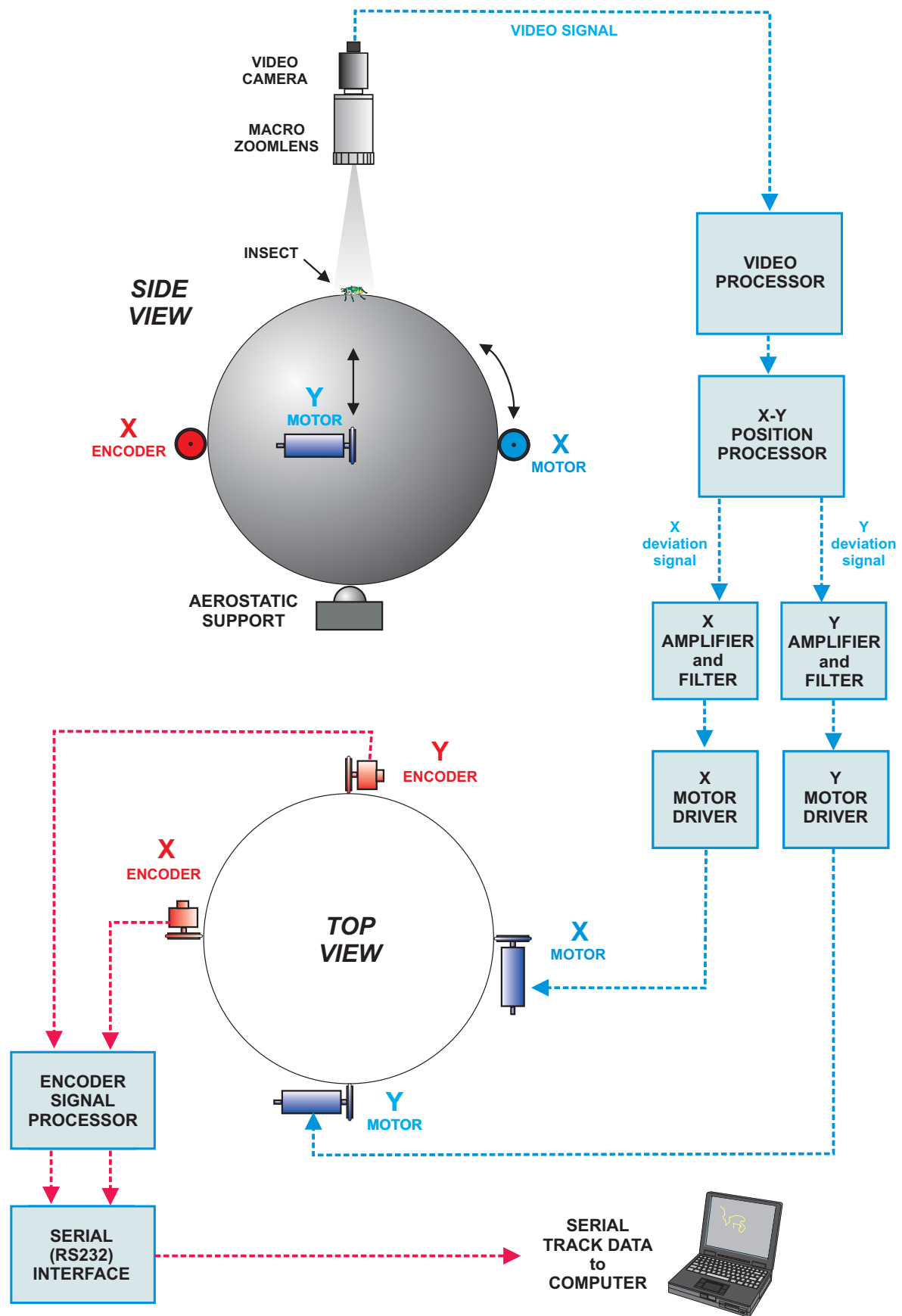
LC-300 with Control Unit and portable PC

LC-100 with Control Unit and portable PC

THE SYSTEM

The complete LC-300 system consists of the following components:

1) Sphere Unit: Sphere
                       Aerostatic support
                       X and Y motors
                       X and Y encoders
                       Sensor and light source support
                       Side mount support

2) Control Unit: Power supply
                       Video display
                       Signal conditioning electronics
                       Feedback adjustment (Gain and Filter)
                       Light source power supply and regulator
                       Data output (RS232 serial connection)
                       Event status inputs
                       Air pump for the aerostatic support

3) Sensor: Video CMOS camera with Macro zoom lens

4) Light Source: IR and Visible LED illuminators

5) Supports: Mounting bracket for stimulus tube
                       Mounting bracket for side-view camera

6) Software   The 'TrackSphere' program collects and stores
                       the displacement data, reconstructs the walking path
                       and provides track analysis

LC-300 PRINCIPLE OF OPERATION

VIDEO CAMERA

MACRO ZOOMLENS

VIDEO SIGNAL

INSECT

SIDE VIEW

Y MOTOR

X ENCODER

X MOTOR

AEROSTATIC SUPPORT

VIDEO PROCESSOR

X-Y POSITION PROCESSOR

X deviation signal

Y deviation signal

X AMPLIFIER and FILTER

Y AMPLIFIER and FILTER

X MOTOR DRIVER

Y MOTOR DRIVER

Y ENCODER

X ENCODER

TOP VIEW

X MOTOR

Y MOTOR

ENCODER SIGNAL PROCESSOR

SERIAL (RS232) INTERFACE

SERIAL TRACK DATA to COMPUTER

PRINCIPLE OF OPERATION

SERVO SYSTEM

Operation of the TrackSphere is based on a servo system, in which an action is counteracted by a reaction through a feedback loop.
The *action* is the displacement of the object (insect or other walking organism) and the *counteraction* is the movement of the sphere in such a way that the action of the object is compensated*: locomotion compensation.*

CLOSED FEEDBACK LOOP

The servo system forms a closed feedback loop: no external influences are relevant for the compensation, only the displacement of the object determines the counteraction, and input (displacement of the object) and output (rotation of the sphere) are directly coupled.
The feedback loop consists of the following elements:

1. The image capturing system: Macro zoom lens and Video camera.
2. The displacement analysing system comprising the Video and X-Y Position Processors.
3. The Amplifiers and filters for the X and Y displacement signals.
4. The X and Y motor drivers.
5. The surface of the sphere.

TUNING THE FEEDBACK

The feedback action is *proportional*: a larger displacement of the object results in a stronger counteraction of the sphere. However, the feedback must be well balanced: if the feedback is too strong the counteraction will be too large; if the feedback is too weak, the compensation will be too slow. The degree of feedback is dependent on, and adjustable by:
 1) The zoom factor of the macro zoom lens (adjustable at the lens)
 2) The amplification of the X and Y deviation signals (adjustable on the control unit).
Both factors must be adjusted such that the compensation is sufficient to keep he object in place at the top centre of the sphere. Too high feedback results in oscillation; too low feedback in a delayed compensation.

FILTER

The *filter* prevents the feedback from acting too fast, or to respond to irrelevant movements of extremities of the object. Always keep the filter setting as low as possible, as the filter may induce delayed feedback or unwanted oscillation.

DATA TRANSFER

Note that the X and Y rotation of the sphere, as recorded by the X and Y encoders, is NOT part of the feedback system. The data of the encoders is independently transferred to a computer via a serial (RS232) connection.

PREPARING THE SPHERE UNIT 1

Mounting the side attachement

Insert air support ball

Placing the sphere

Mounting the support bar

Placing the cover plate

ASSEMBLY

CABLES and TUBES:

Connect the cables and tube as indicated at the receptacles:

> * Power cord
> * Sphere unit interface cable
> * Light source
> * Serial interface cable to RS232 port of computer
> * Air tube

SPHERE UNIT

AEROSTATIC SUPPORT

The aerostatic support is completed by placing the 60 mm ball in the spherical air support cup.
Inspect the air cup support for detrimental particles before inserting the ball.

SPHERE

To avoid contamination in critical experiments it is recommended to use gloves when cleaning and handling the sphere. The sphere is best cleaned with alcohol, rinsed with distilled water, and wiped to dry with tissue.

SENSOR and LIGHT SOURCE:

> * Fix the mounting bar in the clamps at the rear of the sphere unit
> * Attach the CMOS sensor with zoom lens using the double clamp
> * Mount the ring light at the front of the zoom lens

For most applications the macro video lens supplied with the system is the most suitable lens as it allows convenient adjustment of object size and focusing. However, other lens systems may be used.
Adjust the vertical position of the sensor to leave a distance of about 25 cm between the top of the sphere and the lower side of the lens. This distance can be adjusted later depending on the size of the insect.
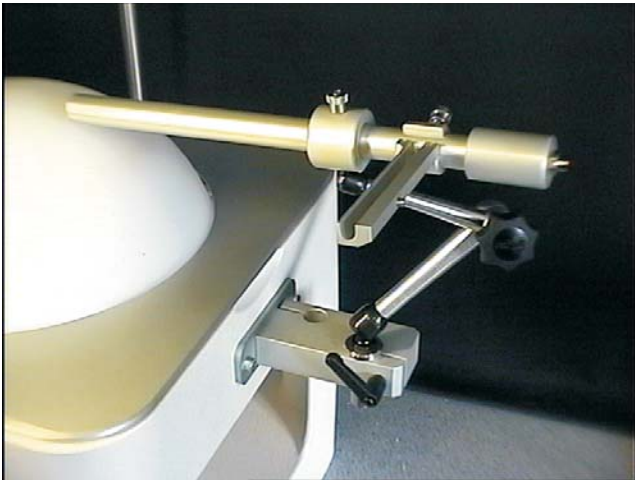It is important to adjust the horizontal position of the sensor to be aligned with the centre of the sphere and to carefully adjust the sensor-lens combination along the vertical axis.

LIGHT SOURCE

The ring light source is attached to the front of the zoom lens.
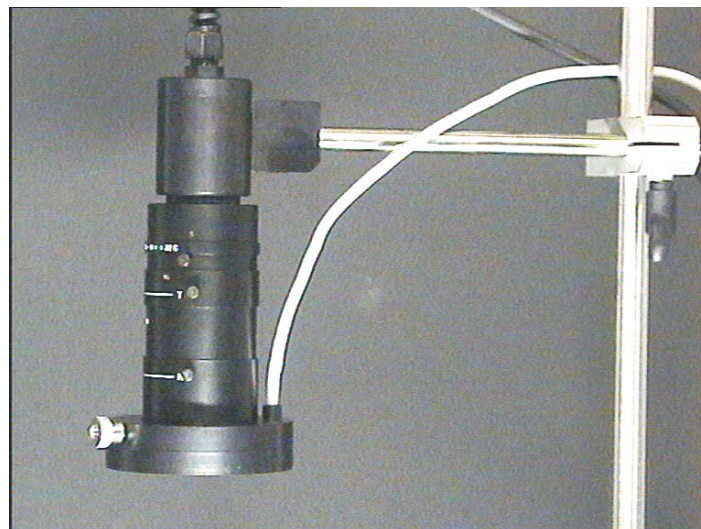The power plug is connected to either the receptacle on the sphere unit or that on the control unit.

Mounting the flow tube
and stimulus applicator

CMOS sensor and zoom lens

Mounting the ringlight

Sphere unit rear panel connectors

SIDE MOUNTING CLAMP

A mounting clamp can be attached to one of the sphere sides using the (short) M6 size screws supplied. *Do not use longer screws; they may damage the mechanism inside the unit*
The clamp can hold two mounting brackets for accessory equipment.

FLOW TUBE and STIMULUS APPLICATOR

   * Attach the mounting clamp to the side of the sphere unit using
     the 2 screws (M6) provided.
   * Insert the swivel brackets for the flow tuber and/or camera mount
   * Slide the stimulus applicator on the flow tube

The flow tube is designed to provide a directed non-turbulent air flow to the object. The inlet socket is supplied with a flow diverter. The tube has an aperture at the side to insert the tip of a Pasteur pipette odour source.
The stimulus applicator is clamped on the flow tube at the position of the aperture. It is designed to hold and easily exchange a Pasteur pipette odour source. Its position with respect to the aperture is adjusted to let the tip of the Pasteur pipette slide into the aperture.

SIDE VIEW CAMERA BRACKET

A suitable bracket to hold a side view video camera can be clamped in the side clamp. The use of a side view video camera is advantageous for recording high quality and detailed video images from a preferred viewing angle.
Although the signal from the CMOS displacement sensor is available from output 20, the resolution of the image is relatively low, and the only available as PAL composite video signal.
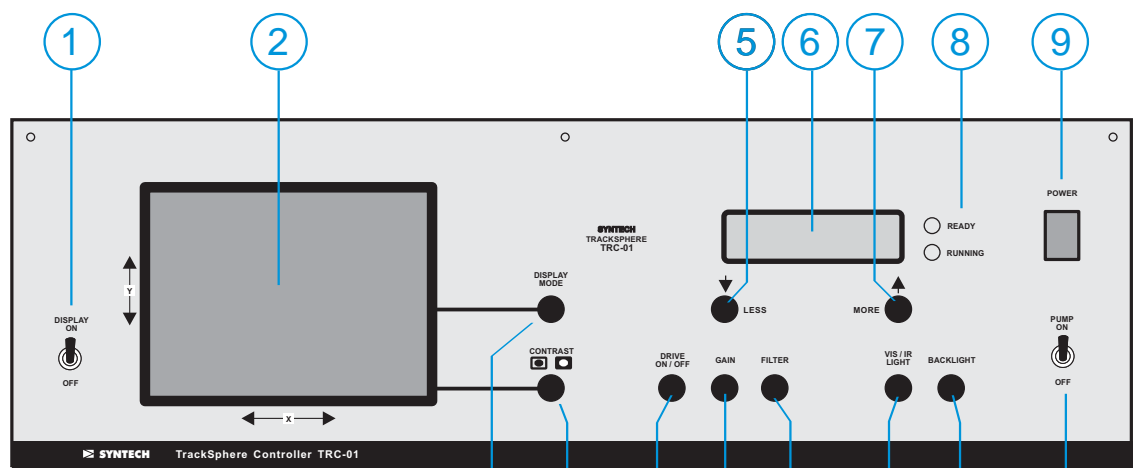
COMPUTER SYSTEM

Any office computer or portable PC running Windows (98 or later) and provided with a serial communication port (RS-232) can be used to record the track data, to display a real time reconstruction of the walking path and to perform track analysis.
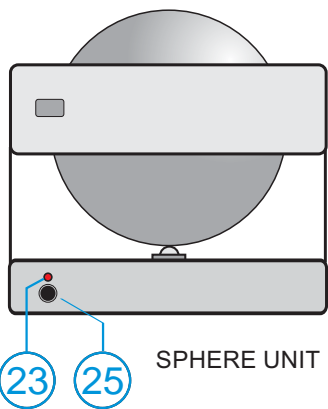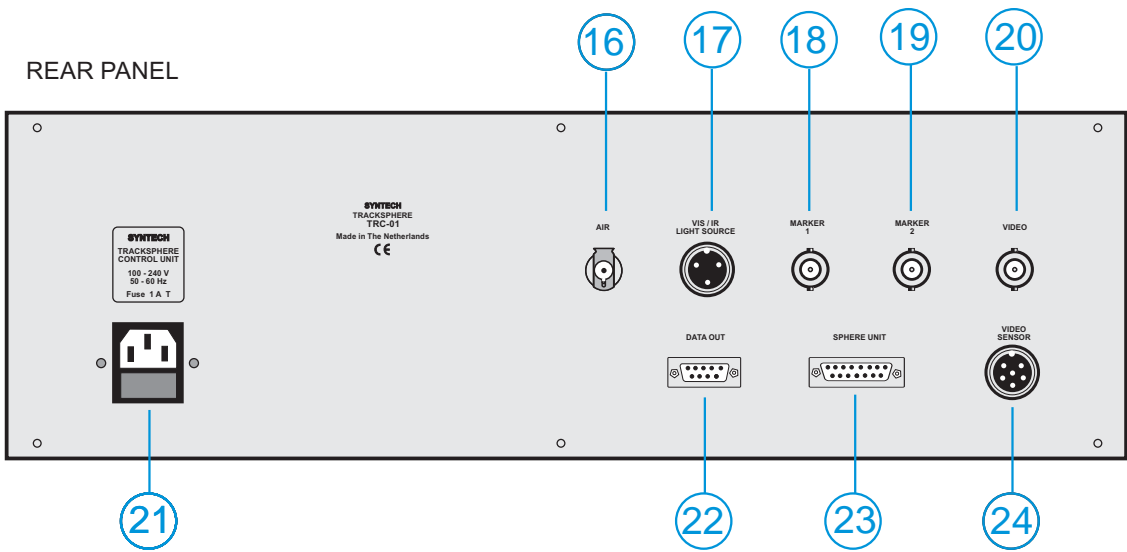
SOFTWARE

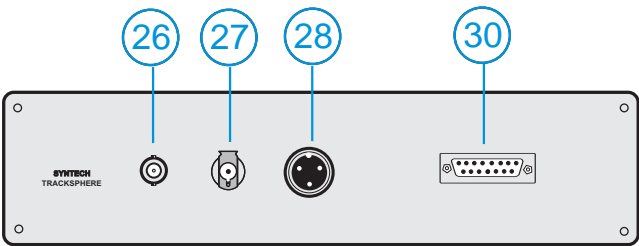The software is installed by running the 'Setup.exe' file on the program CD ROM.

LC-300 CONTROL FUNCTIONS and RECEPTACLES

FRONT PANEL

REAR PANEL

SPHERE UNIT

SPHERE UNIT REAR PANEL
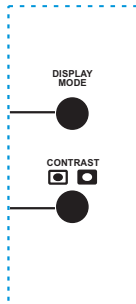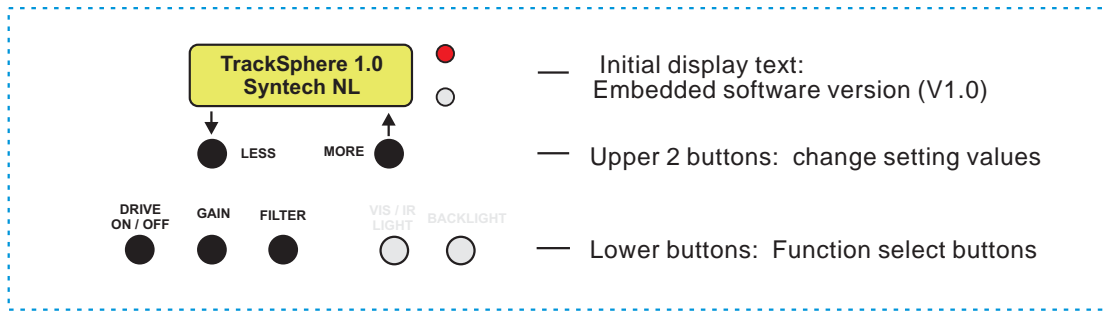
CONTROLS, RECEPTACLES and INDICATORS

CONTROL UNIT

Nr.	Element	Function
1	Display switch	Controls display ON / Off
2	Video display	Shows object in normal video and sensor mode
3	Display Mode	Switches between video and sensor display mode
4	Contrast mode switch	Selects contrast mode (normal / reverse):
		1) Dark object on light sphere
		2) Light object on dark sphere
5	Decrease button	To decrease numerical display value
6	Settings display	Indicates settings and adjustable values
7	Increase button	To increase numerical display value
8	Status indicators	Upper LED: system ready when ON
		Lower LED: system running (feedback active)
9	Power switch	Mains power switch
10	Drive ON/Off switch	Switches motors (and feedback) ON / Off
11	Gain	To set feedback gain (indicated on display)
12	Filter	To adjust feedback filter (via display)
13	Vis/IR switch	Select Visible or IR to be adjusted (display)
14	Backlight	To adjust display backlight intensity
15	Air pump switch	To switch internal air pump ON / Off
		(when external remote pump is used)
16	Air outlet	To connect air tube to sphere unit
17	Light source output	Provides power for Visible and IR light source(s)
18	Marker No1 input	Connector for marker (No.1) switch or signal
19	Marker No2 input	Connector for marker (No.2) switch or signal
20	Video output	Composite (PAL) video output from sensor
21	Power input	Mains power (100 - 240 V; 50 - 60 Hz) receptacle
		and fuse (1 Amp Slow) holder
22	Data output	Serial data output (9-pin D connector)
		for data transfer using ' null modem cable'
23	Sphere unit connector	Provides interface between control unit and
		sphere unit
24	Sensor input	Receptacle for CMOS video displacement sensor

SPHERE UNIT

Nr.	Element	Function
23	Indicator	Indicates RUN mode (same as 8)
24	Drive ON/Off switch	Switches motors (and feedback) ON / Off
		(same as 10)
26	External ON/Off	Switches motors (and feedback) ON / Off
		(same as 10) using external switch (pedal)
27	Air inlet	Air from pump outlet (16) on control unit
28	Light source output	Provides power for Visible and IR light source(s)
		(same as 17)
30	Control input	Connect to controller (23).

**TrackSphere 1.0
Syntech NL**

⬤ (red)
◯

— Initial display text:
Embedded software version (V1.0)

↓ LESS   MORE ↑

— Upper 2 buttons: change setting values

DRIVE ON / OFF   **GAIN**   **FILTER**   VIS / IR LIGHT   BACKLIGHT

— Lower buttons: Function select buttons
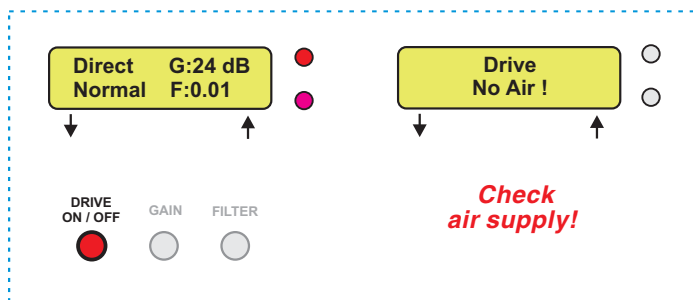

DISPLAY MODE

DISPLAY MODE

Toggle switch between NORMAL video display
and ' SENSOR MODE' video display

CONTRAST

CONTRAST

Contrast mode selector:
1) Dark object on light background (sphere)
2) Light object on dark background (sphere)

**Direct   G:24 dB
Normal   F:0.01**

⬤ (red)
⬤ (magenta)

**Drive
No Air !**

◯
◯

↓   ↑

↓   ↑

DRIVE ON / OFF   GAIN   FILTER
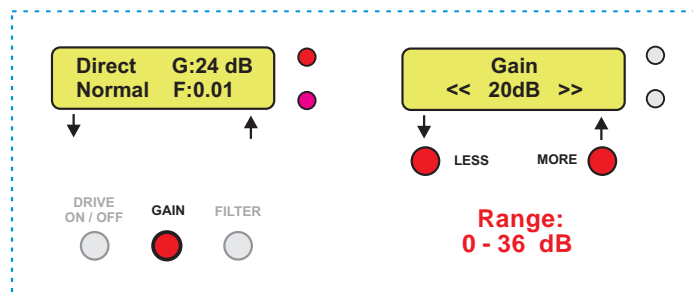⬤(red) ◯ ◯

***Check
air supply!***

DRIVE ON/Off

The sphere motors and the feedback
are activated by pressing this button.
The On/Off button on the sphere unit (25)
has the same function.

If the air supply to the aerostatic support
is leaking or not connected this is indicated
on the display.

**The ' Running' indicator LED is ON when
the feedback is active.**

**Direct   G:24 dB
Normal   F:0.01**

⬤ (red)
⬤ (magenta)

**Gain
<<   20dB   >>**

◯
◯

↓   ↑

↓ LESS   MORE ↑
⬤(red)   ⬤(red)

DRIVE ON / OFF   GAIN   FILTER
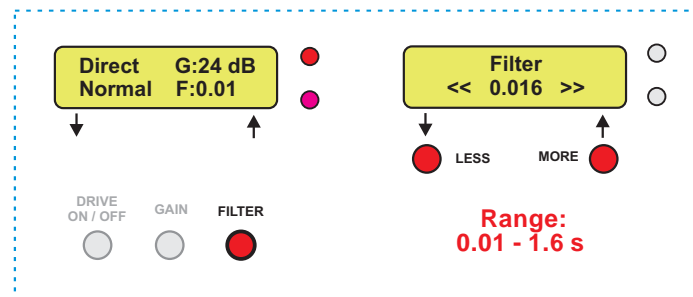◯ ⬤(red) ◯

**Range:
0 - 36 dB**

GAIN

The GAIN of the displacement amplifiers,
and thus the speed of the displacement
compensation must be adjusted to the
walking speed of the object.

**Too LOW a gain resuls in lack of compensation.
Too HIGH a gain results in oscillation.**

The GAIN is also dependent on the zoom factor
as adjeted by the zoom lens. A higher zoom factor
is balanced by a lower gain.

**Direct   G:24 dB
Normal   F:0.01**

⬤ (red)
⬤ (magenta)

**Filter
<<   0.016   >>**

◯
◯

↓   ↑

↓ LESS   MORE ↑
⬤(red)   ⬤(red)

DRIVE ON / OFF   GAIN   FILTER
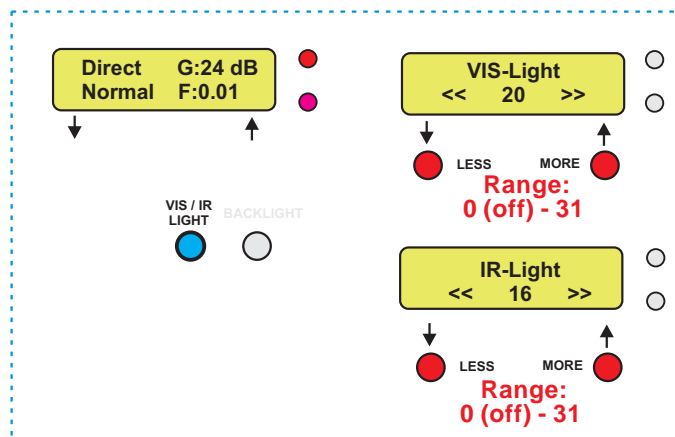◯ ◯ ⬤(red)

**Range:
0.01 - 1.6 s**

FILTER

The feedback amplifiers are supplied
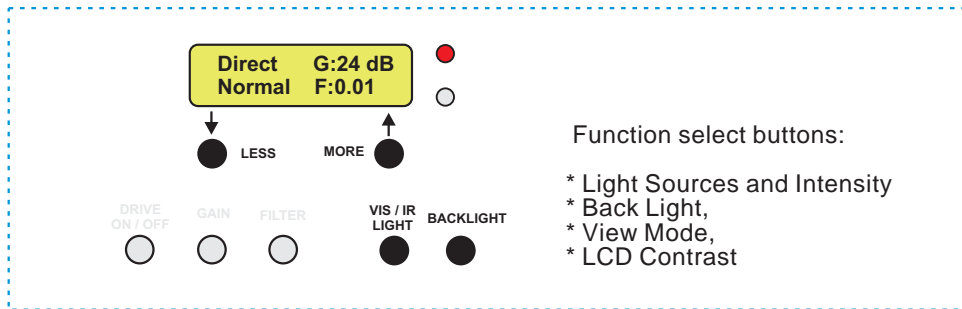with adjustable low pass filters.
The filters prevent too fast displacements
and movements of extremities to result
in unstable compensation.

Do not select a higher filter setting than
is necessary to obtain a smooth compensation

**Too HIGH a filter setting combined with high
gain may result in oscillation.**

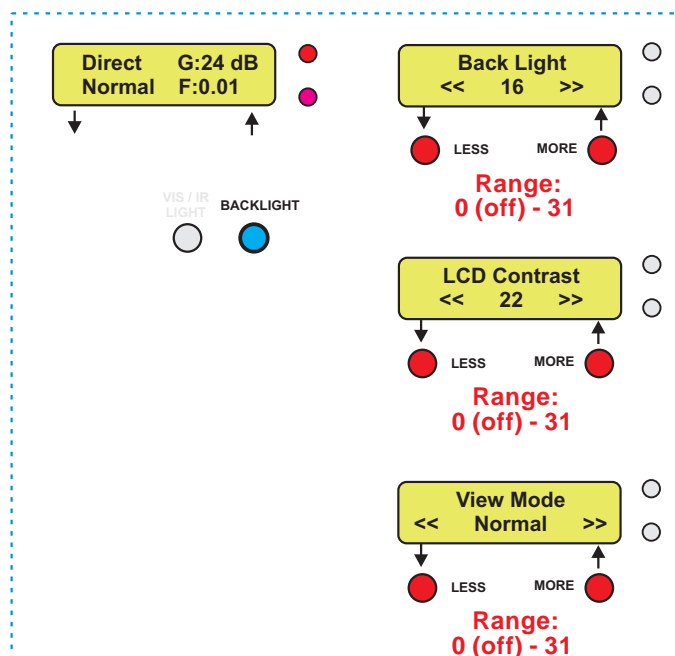If no button is used the display returns to normal after 10 seconds.

Direct   G:24 dB
Normal   F:0.01

LESS   MORE

DRIVE
ON / OFF   GAIN   FILTER   VIS / IR
LIGHT   BACKLIGHT

Function select buttons:

* Light Sources and Intensity
* Back Light,
* View Mode,
* LCD Contrast

Direct   G:24 dB
Normal   F:0.01

VIS / IR
LIGHT   BACKLIGHT

VIS-Light
<<   20   >>

LESS   MORE
Range:
0 (off) - 31

IR-Light
<<   16   >>

LESS   MORE
Range:
0 (off) - 31

VISIBLE
and / or
INFRA RED (IR)
Light Source

For each of the visible and IR light
sources the intensity can be adjusted.
When set to 0 the light source is OFF.

Combined Light sources for Visible and IR
can be connected to output 17 (or 28) or
single mode light sources (Visible or IR)
for increased intensity are available.

Direct   G:24 dB
Normal   F:0.01

VIS / IR
LIGHT   BACKLIGHT

Back Light
<<   16   >>

LESS   MORE
Range:
0 (off) - 31

BACKLIGHT

LCD Contrast
<<   22   >>

LESS   MORE
Range:
0 (off) - 31

LCD Contrast

View Mode
<<   Normal   >>

LESS   MORE
Range:
0 (off) - 31

VIEW MODE

Normal

Direct   G:24 dB
Normal   F:0.01

Tracking

X: 257   dX: 4
Y: 165   dY: 2

Place
cover plate
if required

Iris
diaphragm

Zoom
adjustment

Locking screw

Focus
adjustment

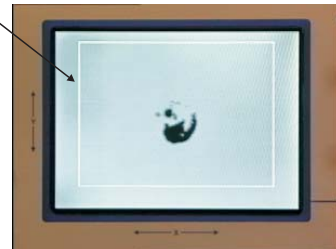ZOOM LENS

Place
piece of paper
with
black spot
on top of sphere

White RECTANGLE
deliniating
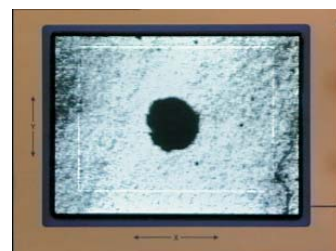sensor field

GOOD
contrast

Normal
video image

Switch to
display
SENSOR IMAGE

DISPLAY
MODE

OVER
illuminated

UNDER
illuminated

OPERATION 1

INITIAL SETTINGS

* Run/Inhibit on control unit: ON
* Run/Inhibit on SPHERE unit: Off
* Filter: 0.3
* Gain: 4
* Display mode: Normal Video
* Contrast mode: Dark/Bright (Up)
* Air pump: ON (don't forget!)

CENTERING and FOCUSSING

* Carefully insert the sphere (do not forget to place the 60 mm ball in the aerostatic support).
* Place a piece of paper with a black dot on the top centre of the sphere.
* Check all cables and the air tube for proper connection. Switch the system on; switch the video display on (press the appropriate button).
* Open the iris diaphragm fully; adjust the zoom factor, and focus on the black dot while watching the image of the spot on the display.
* Align the sensor position, the zoom factor and focus adjustment to achieve a proper image of the dot in the centre of the video screen.
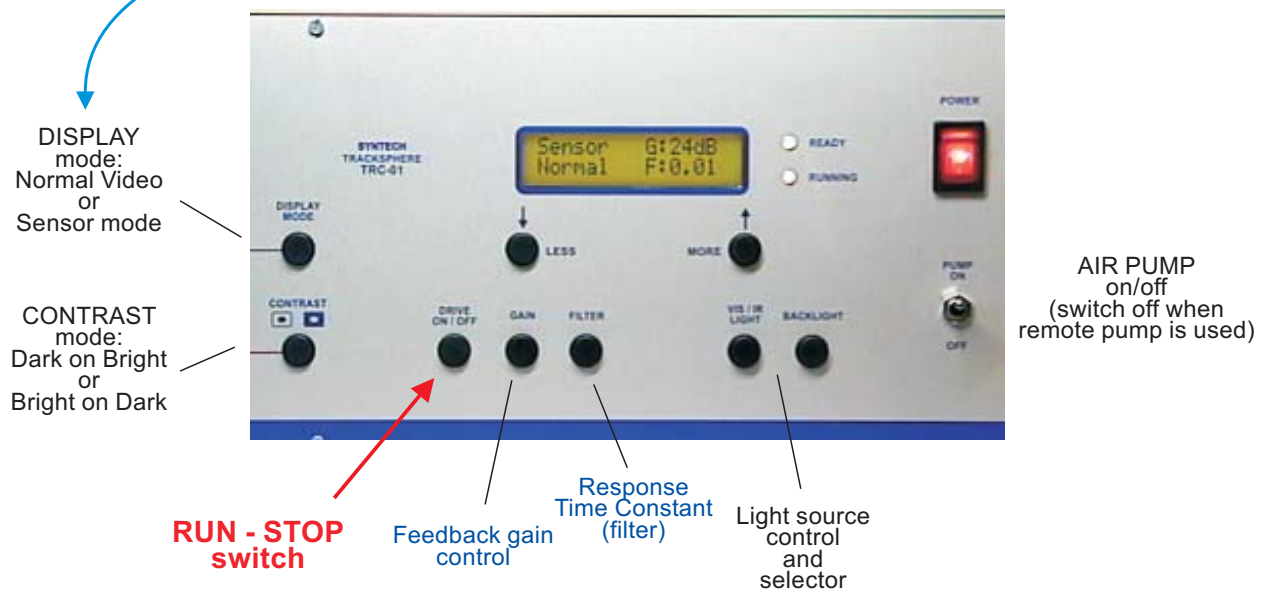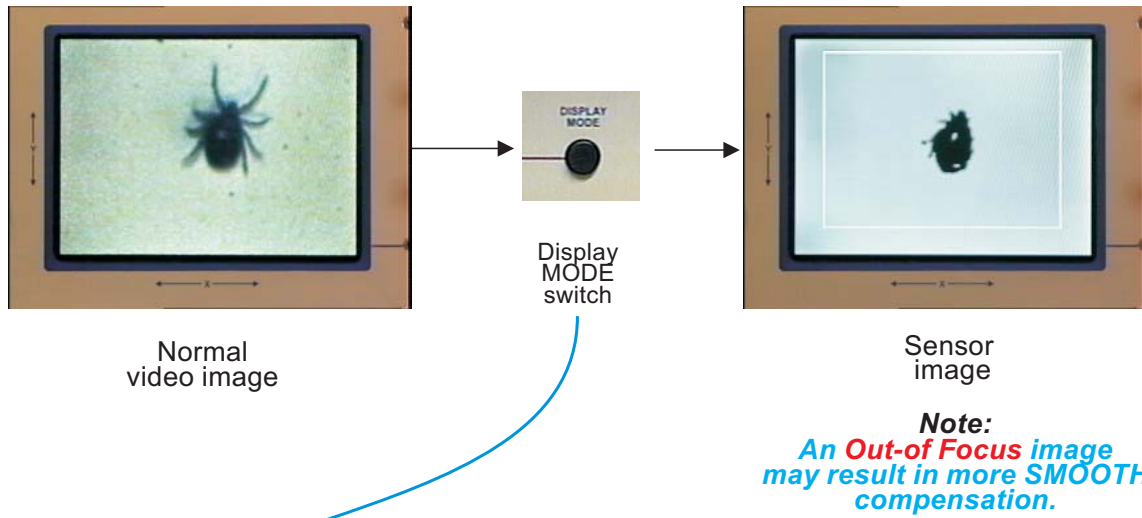
ILLUMINATION and CONTRAST

Operation of the displacement sensor is based on the contrast between the insect and the surface of the sphere. Therefore, the illumination must be adjusted properly to achieve the necessary contrast. The contrast can be evaluated on the video display in the sensor mode. In this mode the display shows the signal from the displacement sensor; the sensitive area is delineated by a white rectangle.
* switch to sensor mode and adjust the illumination to optimise the contrast.
* The illumination can be adjusted by:
        1) the iris diaphragm of the lens
        2) the intensity of the ambient light
        3) the intensity of the light source
* A proper contrast is characterized by a well defined spot in a clear area.
* The size of the spot and its 'structure' are not important.
* It is not necessary to focus very precisely.

TEST

Switch the sphere unit to RUN and gently slide the piece of paper over the top of the sphere: the sphere should respond with an opposite rotation.

Normal
video image

Display
MODE
switch

Sensor
image

Note:
***An Out-of Focus image
may result in more SMOOTH
compensation.***

DISPLAY
mode:
Normal Video
or
Sensor mode

CONTRAST
mode:
Dark on Bright
or
Bright on Dark

**RUN - STOP
switch**

Feedback gain
control

Response
Time Constant
(filter)

Light source
control
and
selector

AIR PUMP
on/off
(switch off when
remote pump is used)

**RUN - STOP
switch**

OPERATION 2

After the position of the displacement sensor, the illumination and contrast have been properly adjusted using the dark spot on a piece of paper, the instrument is ready for adjustment of the dynamic parameters using a suitable test insect.
If the size of the insect is substantially different from the size of the test spot applied in the set-up procedure it is recommended to re-adjust the system using a spot of about the same size as the test insect.

* Prepare the system as described in the previous section
* Place the test insect on top of the sphere
  This can be facilitated by:
        1) temporarily immobilization by cooling
        2) anaesthetizing the test insect by CO_2
        3) release from an upside-down vial
* As soon as the insect is well positioned on the video display:
  immediately switch to RUN pressing either the RUN/STOP button on the control unit or the same on the sphere unit.
* Watch the insect on the video screen
* Use the display mode switch to check the sensor output signal contrast quality; if necessary, adjust the illumination .
* Observe the movement of the insect and the response of the sphere
* Adjust the feedback controls:

GAIN: Slow insects require a low gain setting; for fast moving insects the gain needs to be increased.
Too high a gain may result in irregular compensation and causes ***oscillation.***
Always keep the gain *just below the level at which oscillation occurs*.
Always start with a moderate gain value.

FILTER: The filter prevents the sphere from abrupt responses to small displacements of the insect and from responses to movements from extremities.
A high filter setting strongly damps irregular responses of the sphere and smoothes the rotation.
Too high a filter value may result in ***delayed response*** of the sphere or even in ***oscillations***.
Always begin with a low filter value.
Re-adjust the GAIN and FILTER setting while the system is running.
Check the proper contrast picture of the sensor signal (switch to Sensor mode) from time to time and adjust the illumination.
It is important to keep the illumination unchanged during an experiment.

TRACK DISPLAY and RECORDING 1

START
recording

CLEAR
current screen

DISPLAY
options

Untitled - TrackSphere

File   View   Zoom

Viewing   206:33

-1500   -1000   -500   0   500   1000   1500

-800

-400

File   View   Zoom

New   Ctrl+N
Clear the screen

Open...   Ctrl+O
Save   Ctrl+S

File Comment

Properties
Simulate

Print...   Ctrl+P
Print Preview
Print Setup...

About TrackSphere...
Exit

Time: 206:33   location: x=0, y=0   distance: 0 mm   Last: x=0, y=0

ELAPSED   LOCATION   DISTANCE   LAST
time   from origin   displacement

PROPERTIES BOX

TrackSphere Configuration

Com Settings
Serial Port   COM1
                     COM1
                     COM2

Color Settings
Item   Color preview

background color

Draw Settings
                     Line width
☑ Draw Arrow
☑ Draw Ruler
                          4

OK   Cancel

serial
COMMUNICATION
port

COLOR
selection

ITEM
select

LINE
width

RECORDING

After the system has been well prepared for recording, click the RECORD button to start data acquisition. The status indicator changes from green to red showing the elapsed recording time. Stop the recording by clicking the same button.

Saving the recording

After the recording has been finished, the display returns to the' view' mode. Select 'Save' from the file menu; Enter the path and the file name; the files are automatically are given the extension .csv (Comma Separate Values)

The file format is compatible with Windows EXCEL and many other data analysis programs.

The data file consists of 4 columns:

1. Status of event input 1 (active if 0)
2. Status of event input 2 (active if 0)
3. X incremental values in 0.1 mm
4. Y incremental values in 0.1 mm

There are 10 rows of data per second, representing the fixed data sampling rate of the system (10/s). The first row represents the START of the recording at time $= 0$.

DATA ANALYSIS

The present TrackSphere software (Version 2.2 ; Sep. 2003) does not provide additional data analysis tools. However, in the appendix of this manual EXCEL routines developed by Dr. J. Hans Visser (Plant Research International, Wageningen,The Netherlands) are presented for detailed calculation of relevant parameters during a test run.
Data collected by the TrackSphere program can be processed by the EXCEL routines provided, and information is presented on how to handle the raw data generated by the Syntech TrackSpheres.
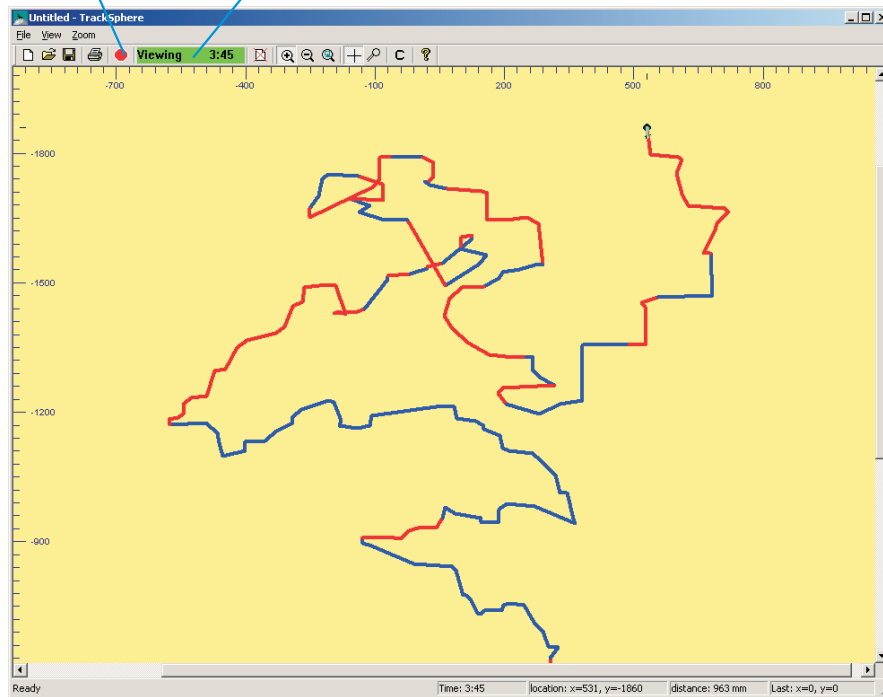The EXCEL routines are available on the software CD (TRACKanalysis.xls). These routines may also serve as examples for the development of specific routines by the user of the TrackSphere system.

The routines developed by Visser will be implemented in a future upgrade of the Syntech TrackSphere program to form an integral part of the program. The upgrade is expected to be available from the Syntech website (www.syntech.nl) by August 2004.

TRACK DISPLAY and RECORDING 2

START / STOP recording

Elapsed time and Status Indicator

EVENT MARKER 1 and 2 INPUT STATUS
indicated by color of track

	Event 1	Event 2	X	Y

EXCEL data

	A	B	C	D	E
10	1	2	0	0	
11	1	2	0	0	
12	1	2	0	0	
13	1	2	0	0	
14	1	2	0	0	
15	1	2	0	0	
16	1	2	-1	0	
17	1	2	1	0	
18	1	2	0	0	
19	1	2	89	-89	
20	1	2	132	-40	
21	1	2	136	-44	
22	1	2	124	-39	
23	1	2	130	-40	
24	1	2	132	-42	
25	1	2	130	-41	
26	1	2	131	-43	
27	1	2	135	-44	
28	1	2	129	-40	
29	1	2	131	-42	
30	1	2	133	-41	
31	1	2	130	-41	
32	1	2	131	-40	
33	1	2	126	-37	
34	1	2	134	-36	
35	1	2	129	-39	
36	1	2	132	-40	
37	1	2	136	-39	
38	1	2	132	-39	
39	1	2	128	-38	
40	1	2	132	38	

DATA TRANSFER

The incremental displacements of the insect are measured by the two rotary encoders contacting the sphere at orthogonal positions. The displacements are measured at a rate of 10/s with an accuracy of 0.1 mm.
The 'TrackSphere' software takes care of data transfer via a standard RS232 serial port of the computer.

Software Installation

The TrackSphere software is delivered on a CD or can be downloaded from the Syntech website (www.syntech.nl).
Install the software by running the file Setup.exe in the list comprising the installation files.

Running the software

Prepare the TrackSphere system and make the connection with the computer using the serial interface cable (' null modem' cable).
Start the TrackSphere program and switch on the power of the control unit.
If the communication runs correctly an arrow in the center of the screen indicates the position and movements of the sphere.
In case no arrow appears, open the Properties box in the file menu. Select another COM port until the arrow is shown. It might be necessary to switch the control box on and off the start the communication.

The program starts in the 'view' mode: the tracks are displayed real-time, but the data are NOT recorded. The TrackSphere the software shows the displacements of the sphere in real-time mode.
    The data displayed are:
* A real-time display of the reconstructed movement path
* The X and Y positions in 0.1 mm relative to the starting point
* The total distance moved in mm.
* The time elapsed since the last Record or Restart command
* The status of the two event inputs

Make any required modifications in the appearance of the screen using the settings in the Properties box.

Event Markers

The 2 event marker inputs (BNC receptacles 18 and 19 at the rear of the control unit) can be used to indicate and record any change in the stimulus environment of the object. The event marker changes (from 1 to 0) when the input is shorted by means of a switch or by a TTL command from a stimulus device.

MAINTENANCE

SPHERE

Always remove and insert the sphere carefully to prevent it from scratches.
The sphere can be cleaned with water or alcohol using soft tissue; rinse
with distilled water.
For contamination-critical experiments it is recommended to handle the sphere
with gloves.

AEROSTATIC SUPPORT

As the 60 mm ball in the aerostatic support may take up contamination from the
surface of sphere it should be cleaned from time to time. Cleaning is best done
with water or alcohol.
Before replacing the ball inspect the support for detrimental particles and dust,
which should be removed carefully with a soft tissue.

MOTOR and ENCODER FRICTION RINGS

Contamination from the surface of the sphere may be collected on the rubber O-
rings
of the drive pulleys on the motor and encored shafts.
The O-rings can be easily removed, cleaned in alcohol, and replaced.
After some time the O-rings must be replaced.
The O-rings are made of silicone rubber (hardness: 70o shore), and their size is:
24 x 2.5 mm.

ACCESSORIES

EXTERNAL AIR PUMP

For sound and noise-critical experiments the internal pump in the control unit can
be switched off. To supply the necessary air an external pump, which can be
placed outside the experimental area, is available from Syntech.

PEDAL SWITCH

To keep both hands free for initial positioning of the object a pedal switch
operating the ON/Off command is advantageous. It is connected to the BNC
receptacle (26) at the rear of the sphere unit.

STIMULUS DELIVERY DEVICES

A range of programmable stimulus controllers for delivery of single odours and
mixtures is manufactured by Syntech. A programmable multi-channel dynamic
olfactometer is currently under development. Stimulus generators for other
modalities (sound, radiation, vibration, light etc.) are constructed on user
specifications.

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.
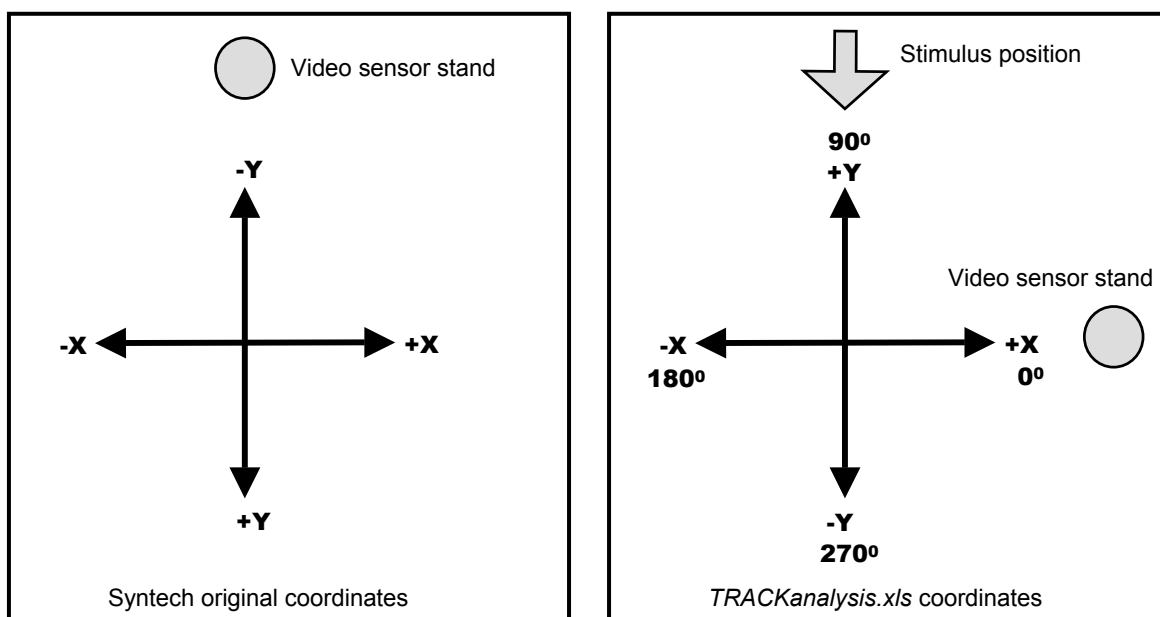
- **Walking speed** is track length per s in mm/s, graphically shown in time.
- **Average speed** is average of walking speeds (including zero speeds) in mm/s.
- **Standard deviation of speed** is standard deviation of average speed (n-1 method) in mm/s.
- **Track length** is the total length of the 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.

Syntech original coordinates	*TRACKanalysis.xls* coordinates

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 behaviour, 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 behaviour. 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 odours, mated females may not respond to sex pheromones, or prior experience with food, followed by starvation, increases insect responses towards food odours. 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 analysed **statistically** and do not trust your own capabilities just looking at walking tracks.

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