

INSTALLATION AND RUNNING THE SOFTWARE

1 Download the latest version of LoliTrack v5 from our website: www.loligosystems.com/downloads Follow the instructions on the screen and then restart the PC.

2 Connect the Loligo® license dongle to a USB port on the PC to unlock the full software (2).

RECORD VIDEOS FOR 2D TRACKING

3 Consider using **Video Recorder** if you need to record videos for LoliTrack v5. Video Recorder is our free video recording and editing software designed for use with Loligo® cameras. The software also accepts most modern webcams, but with limited functionality. You can download the software here:

www.loligosystems.com > Resources > Software downloads > Behavior

2D TRACKING

4 Watch the online video tutorial series on how to do 2D tracking in LoliTrack v5. Start with: [LoliTrack v5 - 2D Tracking - How to import videos](#)

Open **Tracking 2D** from the main menu. Click on and choose a video file. Change the **File resolution** (lower resolution = faster tracking), if needed. Enter a number in the **Time-lapse field** to divide the Length [frames] by that number to reduce total amount of frames in video (fewer frames = faster tracking). Click Next to continue (4).

5 In the **Calibration tab**, choose the desired Distance, Time and Angle units. Now drag the X/Y-axes to align with a known length on the video preview (5). Input the actual length of the X-axis (or Y-axis), and click the **Calculate value from X-axis** (or Y-axis) icon (5.1). Applying **Distortion correction**, if needed (instructions in online video tutorial). Click OK to save the calibration. Click Next to continue.

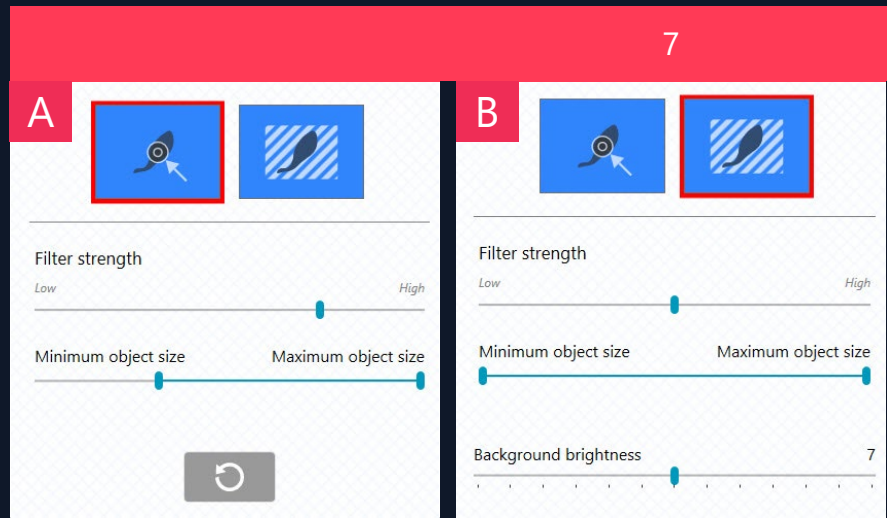
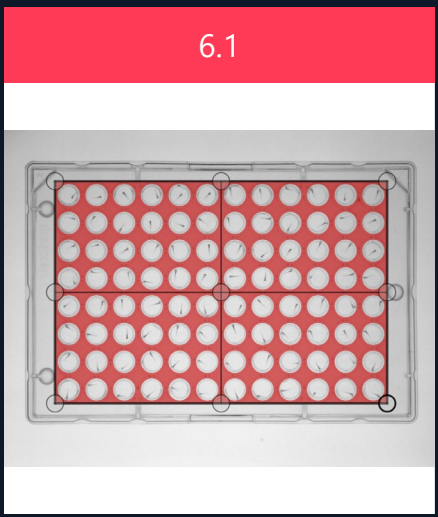
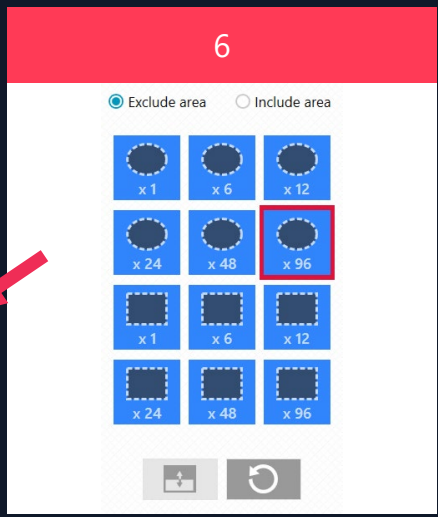
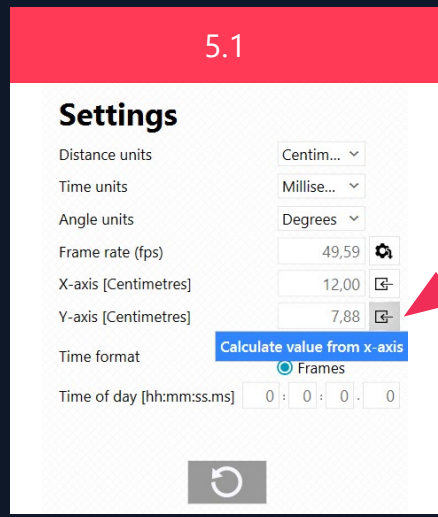
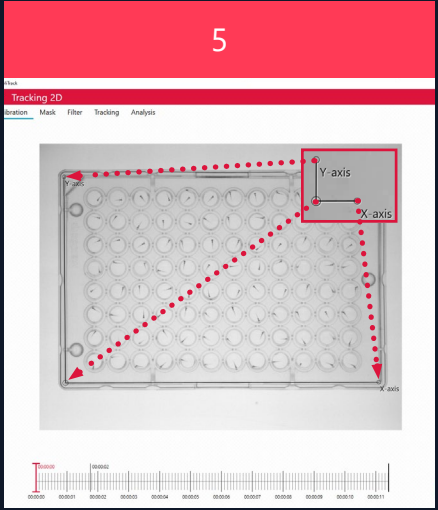
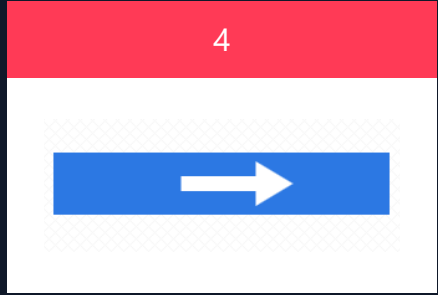
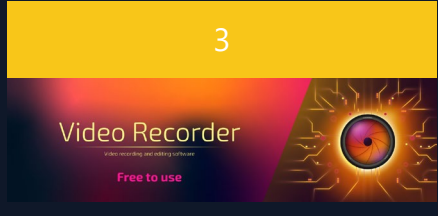
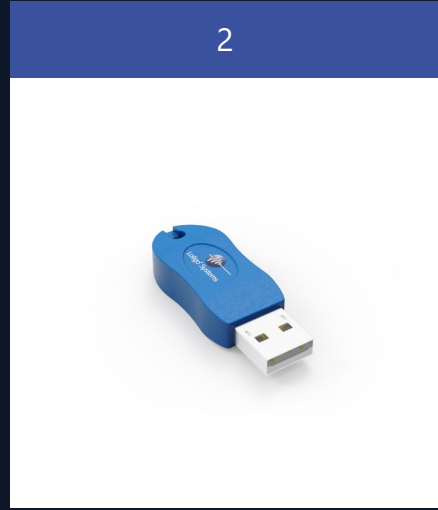
- Additional functions:
- Hold cursor on video preview and scroll mouse wheel to zoom in/out.
 - Drag the video preview around by right-clicking and dragging.
 - Hold cursor on timeline and scroll mouse wheel to zoom in/out.
 - Drag timeline from side to side by right-clicking and dragging.
 - Use arrow keys for frame-by-frame control while keeping cursor on timeline.

6 In the **Mask tab**, use masking tools in Settings panel (6) to mask out unwanted areas by clicking and dragging with selected tool on video preview to create a shape. Adjust the shape using anchor points. Objects in red areas will not be tracked. Every closed, non-masked area defines an arena (i.e. there are 97 arenas in the example (6.1)). Save the mask as a file for later use, if needed. Click OK and Next to continue.

7 In the **Filter tab**, you can create a filter that will threshold images into objects (yellow pixels), that you want to track, and irrelevant objects or background. First select filter mode A (color/contrast) or B (background subtraction):

- A. Threshold objects based on color contrast:**
1. Left-click an object in the video preview to indicate color of the object. Shift + left-click to indicate color of the background.
 2. Adjust *Filter strength* to threshold more selected object pixels. Adjust *Minimum/Maximum object size* to filter out noise (small pixels) or larger objects.
- B. Threshold objects by subtracting static background pixels:**
1. Increase *Filter strength* to threshold out moving objects.
 2. Adjust *Minimum/Maximum object size* to filter out unwanted objects.
 3. Adjust *Background brightness* for improved threshold on bright/dark objects.

Scroll through video for a visual check of the yellow pixels and change settings, if needed. Click OK to save the filter. Click Next to continue.



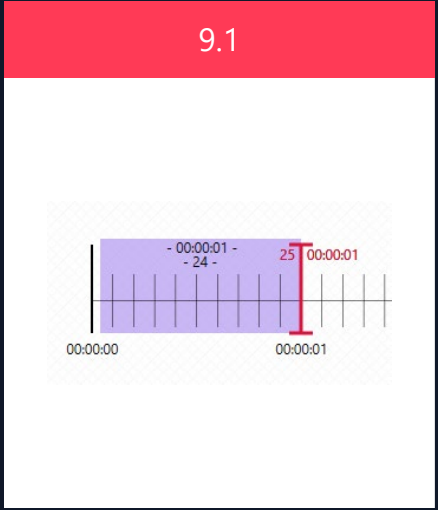
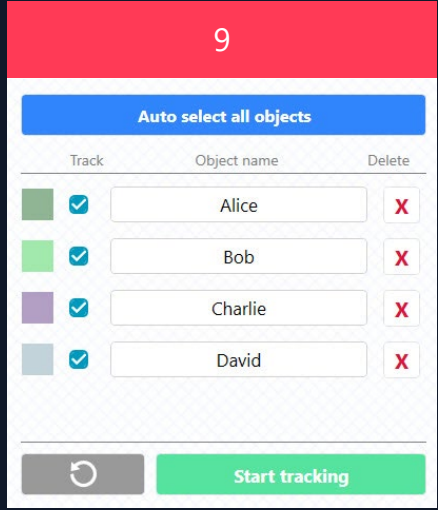
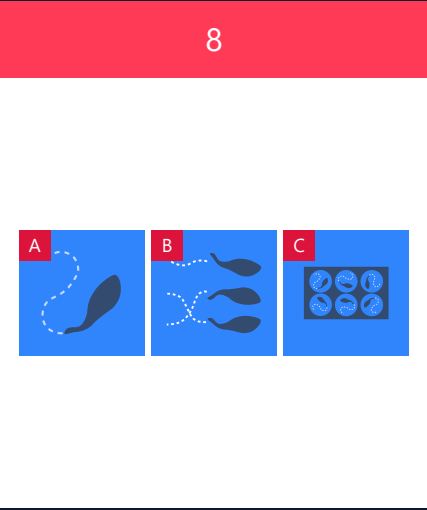
2D TRACKING

8

In the **Tracking tab**, select the best tracking method for your setup (8).

- A. Track a single or a few objects with no occlusions (*when one object is hidden by another object that passes between it and the observer*).
- B. Multiple objects with occlusions.
- C. Multiple arenas with no more than 1 object in each arena.

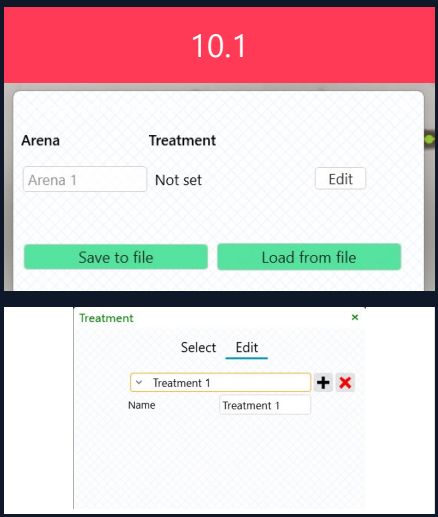
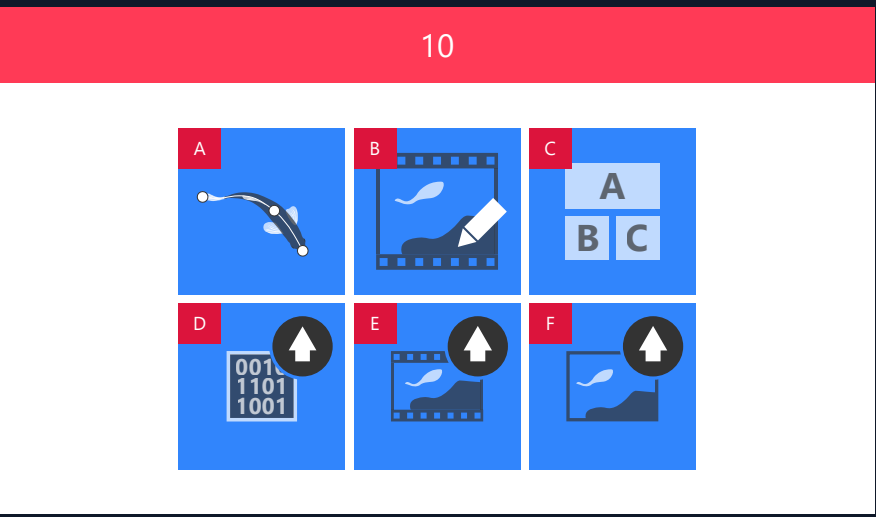
Move time cursor to a point on the timeline where all objects are easily identified (no occlusions or object fusing with background) to help start tracking. The object midline can be tracked, but it increases tracking time. A *Simple* midline is good for e.g. fish, while *Fine* is recommended for worms etc. Choose *None* if a midline is not needed. A midline is needed if you want to track other object position points than the *Center of gravity*.



9

In the **Tracking tab**, click **Auto select all objects** to add all visible objects (yellow pixel clusters) to your object list (9). Alternatively, left-click on each object to add it to the list and right-click to remove. When an object is added to the list, change the color (click color block) and rename it, if needed.

Track entire video by clicking **Start tracking**. Alternatively, select an interval (hold shift + drag) on the timeline (9.1) and then click Start tracking to track only this interval. When the tracking is completed, click Next to continue. Watch video tutorial on how to correct identity switch errors.

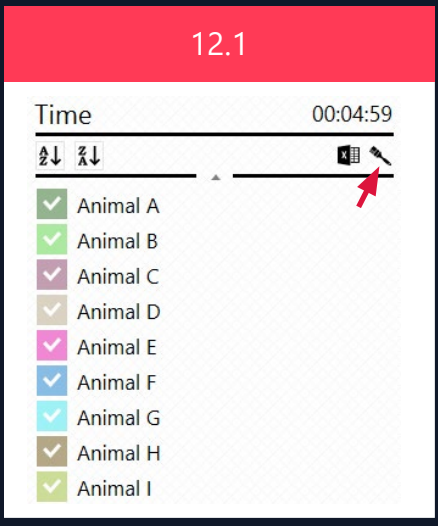
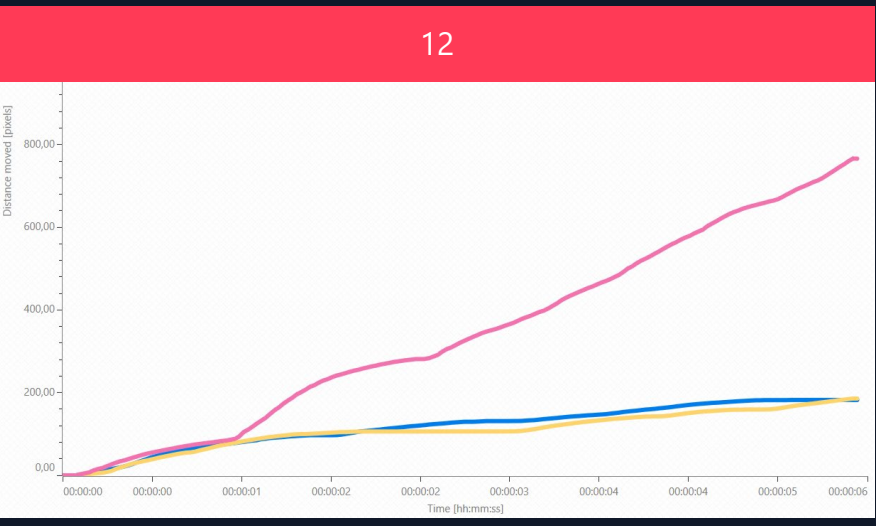


10

In the **Analysis tab**, you can add **Zones** as a video overlay and select **Parameters** before exporting tracking data to Excel or as a media file (10).

- A. **Position settings:** Choose the position point on the object that the tracking data will be based on. Adjust the **Activity-threshold** to remove unwanted pixel-noise. Choose the number of frames over which data averaging is performed (**Smoothing**). **Exported midline points** determines the resolution of the midline.
- B. **View settings:** Adjust which parameters are shown as overlay on the video preview.
- C. **Arenas and treatments:** Edit the names of arenas and treatments, and apply a treatment to a given arena (10.1). The arena names and applied treatments can be saved and applied to a video with an identical setup.
- D. **Export to Excel:** Select the parameters that will be included in the Excel file for the entire video (or interval, if selected on the timeline). The tracking data in the Excel file is based on the selected object position point. Tracking data for arenas, zones and treatments can be exported as well.
- E. **Export video:** Export the entire video (or interval, if selected on timeline) with the overlays shown in the video preview window.
- F. **Export image:** Export the current frame with the overlays shown in the video preview window.

Use the **Batch analysis** to analyze and export data for multiple videos from the same setup. Every setting in the Calibration, Mask, Filter, Tracking, and Analysis for the current video will be applied to the other input videos.



11

Frequency analysis. Main menu > **Frequency analyzer** to find heart rates, tail beats, ventilations, and other types of frequencies from video recordings. You can also do volume calculations to estimate stroke volume, pumping volume, etc. For details on how to use the frequency analyzer, watch the video tutorial:

[LoliTrack 5 - How to use the Frequency analysis tool](#)

12

In the **Analysis tab**, tracking data for the selected object position point is shown in the graph tabs (upper left) for Speed, Acceleration, Distanced moved, Bend, NND, AIID, and MIID. You can export any of the graphs (12) by clicking the **Export to Excel** icon in the Settings panel. Additionally, you can change the color and style of the plotted data by clicking on the **Style** icon in the same panel (12.1, red arrow). The **Bend** graph tab includes a frequency analyzer that uses the data shown in the graph, and is thus useful for analyzing tail beat frequencies.

INSTALLATION

- Download the latest version of LoliTrack v5 from our website: www.loligosystems.com/downloads. Follow the instructions on the screen and then restart the PC.
- Connect the Loligo® license dongle to a USB port on the PC to unlock the full software (2).

RECORD VIDEOS FOR 3D TRACKING

For accurate 3D tracking in LoliTrack v5, the two cameras must be set up similar to the illustrations specified in step 5, see [5].

Connect both cameras to a USB port on your computer. Use the **Video Recorder** software and open *Video Recording*. Select the second camera from the *Sync with* field (3.1). Change frame rate or resolution (using area of interest sliders) in the Settings panel for each camera, if needed. **File frame rate:** The software will attempt to record at this frame rate. **File resolution:** The video will be scaled and saved to this resolution.

- Press **REC** to open the file prompt and start synchronized recording. *Note: A video file from each camera will be saved.*
During recording, four parameters are shown at the bottom of the screen:

- Recorded time:** The length of the recorded file (number of captured frames / file frame rate)
- Current frame rate:** The actual frame rate during recording
- Captured frames:** The number of captured frames
- Skipped frames:** The number of skipped frames

3D TRACKING

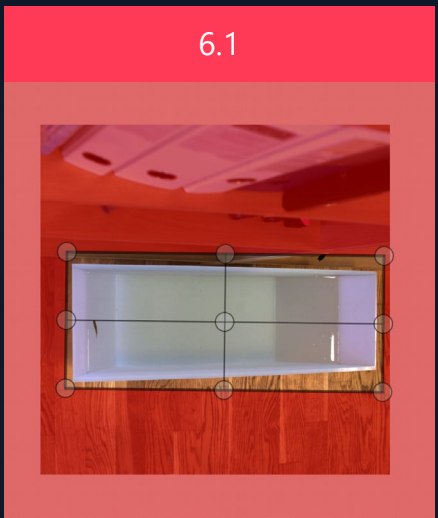
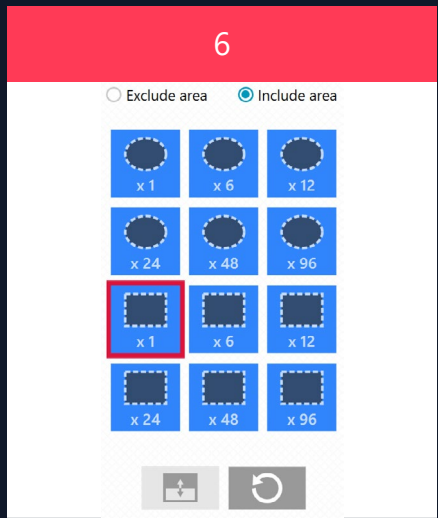
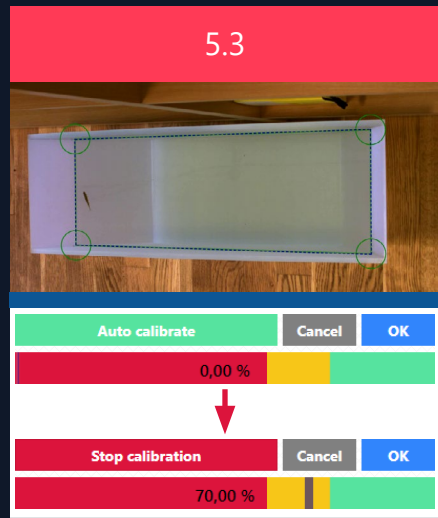
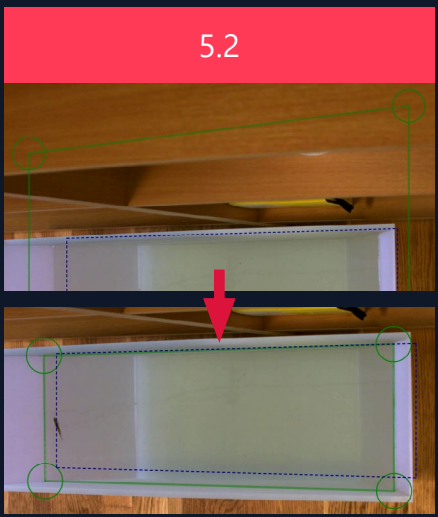
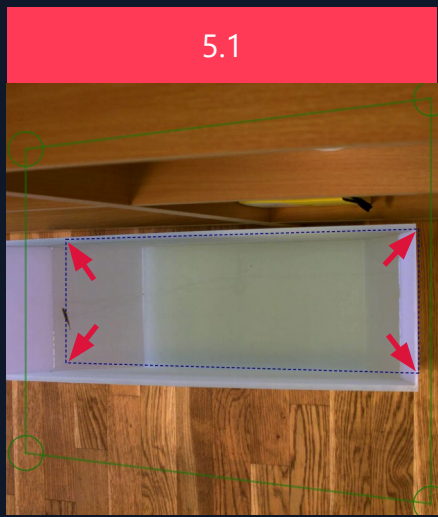
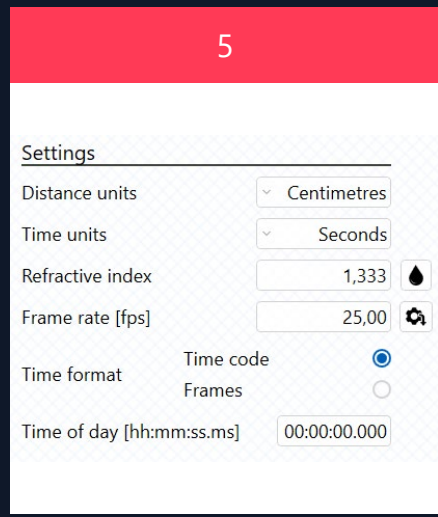
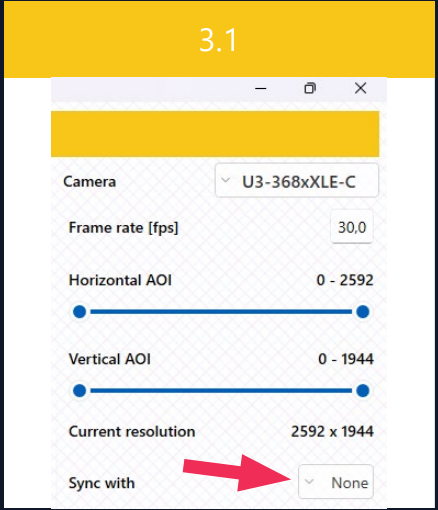
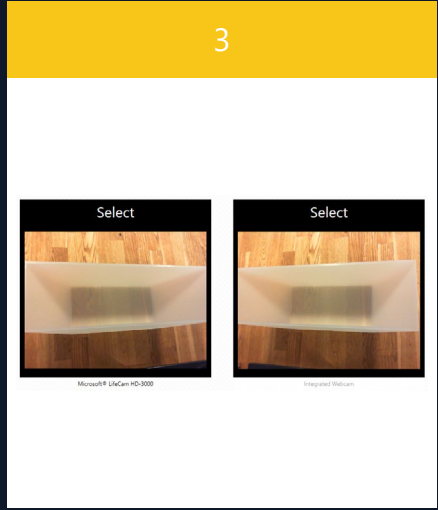
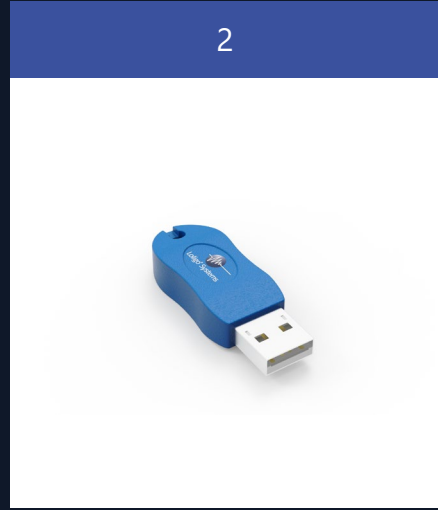
- Open **Tracking 3D** from the main menu. Click on both [5] and choose a video file for each. Change the **File resolution**, if necessary. *Note: 3D tracking is only available for a single object.* Click Next to continue.

In the **Calibration tab**, choose the desired Distance and Time units (5). Specify the **Refractive index** of the medium that the object is in (typically water or air). In the **Parameters section**, fill out the parameter fields (L, W, D... etc.). Click on [5.1] to view illustrations of the camera setup. A blue square with dotted stroke will now appear on the video preview (5.1). Drag anchor points on each green square to the surface defined by L and W (see Setup [5.1]), so that the anchor points mark the corners of the surface (5.2). If the tracked object is not in water, the green square can define the bottom of the chamber.

- Click **Auto calibrate**. The blue square should now start to approximate the shape of the green square (5.3). As the auto calibration process is an approximation, the progression status will move towards 100 % completion (5.3). It is acceptable to stop the auto calibration process, once the status is in the yellow or green area. If the progression status does not enter the yellow or green area, either change the parameters or verify that the blue square is actually on the surface. Click OK to save the calibration. Click Next to continue.

- Additional functions:
- Hold cursor on video preview and scroll mouse wheel to zoom in/out.
 - Drag the video preview around by right-clicking and dragging.
 - Hold cursor on timeline and scroll mouse wheel to zoom in/out.
 - Drag timeline from side to side by right-clicking and dragging.
 - Use arrow keys for frame-by-frame control on timeline.

- In the **Mask tab**, select a masking tool (6) for each camera (cam 1/2 subtab) to mask out any pixels/areas that should not be processed by the software. Use mouse cursor to draw masking shapes on the video preview. Adjust the size and shape using anchor points (6.1). Areas marked with red color will not be tracked/analyzed. Click OK to save mask and Next to continue.



3D TRACKING

7

In the **Filter tab**, change settings to create a filter **for each camera** that will threshold images into objects (yellow pixels), that you want to track, and irrelevant objects or background. First select filter mode A (color/contrast) or B (background subtraction) (7):

- A. Threshold objects based on color contrast:**
1. Left-click an object in the video preview to indicate color of the object. Shift + left-click to indicate color of the background.
 2. Adjust *Filter strength* to threshold more selected object pixels. Adjust *Minimum/Maximum object size* to filter out noise (small pixels) or larger objects.
- B. Threshold objects by subtracting static background pixels:**
1. Increase *Filter strength* to threshold out moving objects.
 2. Adjust *Minimum/Maximum object size* to filter out unwanted objects.
 3. Adjust *Background brightness* for improved threshold on bright/dark objects.

Scroll through video for a visual check of the yellow pixels and change settings, if needed. Click OK to save the filter. Click Next to continue.

7

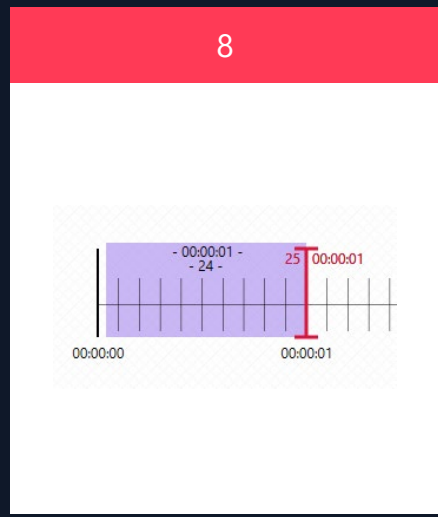
A

B



8

In the **Tracking tab**, chamber depth and height should generally be left empty, but you can enter a value to set a constraint on the tracking. Click **Start tracking** to track entire video. Alternatively, select an interval (hold shift + drag) on the timeline (8) and then click Start tracking to track only this interval. When the tracking is completed, click Next to continue.



9

A

B

C

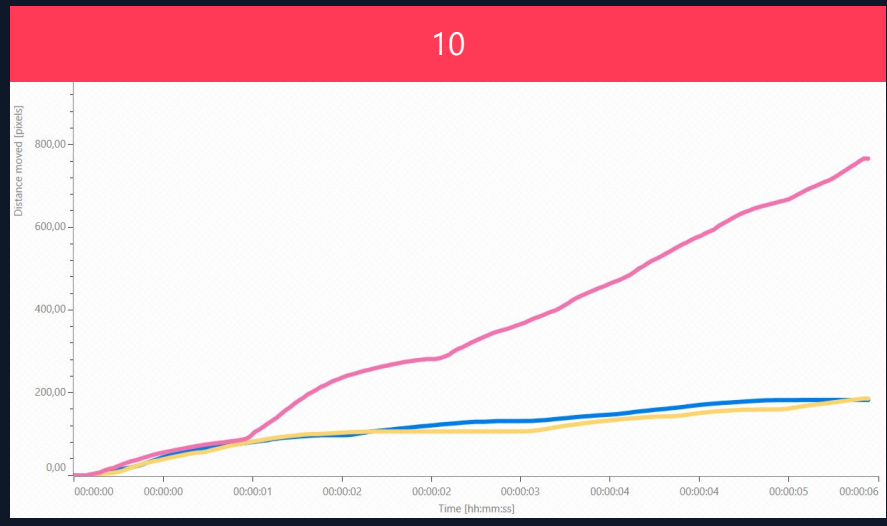
D

9.1

9

In the **Analysis tab**, you can view the 3D tracking and export tracking data to Excel, as a 3D model or as a media file (9).

- A. Export image:** Export the current frame with the overlays shown in the video preview window.
- B. Export video:** Export a 10 seconds video with the overlays shown in the video preview window. In the exported video, the camera will circle once around the 3D model, while the 3D position marker will move along the tracked positions in the selected interval on the timeline. To preview the exported video, click on **Orbit** in the 3D tools (9.1).
- C. Export to Excel:** Export the tracked data to an Excel file for the entire video (or interval, if selected on the timeline).
- D. Export 3D model:** Export a 3D model as either a .glb, .obj, .stl, .u3d file.
- .glb** Can be viewed in most 3D apps on Windows, incl. Word, Excel and PowerPoint.
 - .obj** Standard 3D image format.
 - .stl** 3D image format widely used in 3D printing and modelling interfaces.
 - .u3d** Standard 3D image format. Can be inserted and viewed in PDFs.



10

In the **Analysis tab**, tracking data for Speed, Acceleration and Distanced moved is shown in the graph subtabs (upper left). You can export any of the graphs (10) by clicking the **Export to Excel** icon in the Settings panel. Additionally, you can change the color and style of the plotted data by clicking on the **Style** icon in the same panel (10.1).

10.1

Time 00:04:59

- Animal A (Green checkmark)
- Animal B (Green checkmark)
- Animal C (Purple checkmark)
- Animal D (Brown checkmark)
- Animal E (Pink checkmark)
- Animal F (Blue checkmark)
- Animal G (Cyan checkmark)
- Animal H (Brown checkmark)
- Animal I (Green checkmark)