ZF-Tracker

Novel Tank / Open Field Test Analysis Web App

User Manual

System name: ZF-Tracker
Version: v3.9
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Overview & Purpose

Tool overview

A web app that analyzes zebrafish behavioral data (CSV / .txt files) and automatically calculates behavioral indices. No installation required — runs in your web browser.

Supported tests

NTT (Square tank) and OFT (Square and Circular tanks).

Intended users

Researchers conducting zebrafish behavioral experiments.

Access

Access https://bio-fish-models.com/ from your web browser.

Supported Experiment Types

NTT

Novel Tank Diving Test | Square tank

A test commonly used to assess anxiety-like behavior: diving (bottom dwelling) right after transfer to a novel tank and subsequent exploration of the upper zone [1, 2].

Indices calculated

- Latency to first enter the upper half
- Entries into / time in the upper half
- Total distance and mean speed
- Freezing duration and count

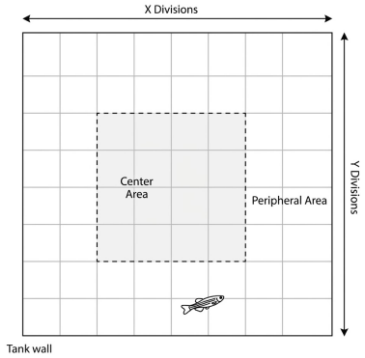
OFT

Open Field Test | Tank shapes: Square / Circular

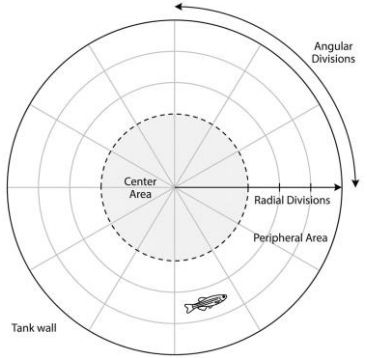
Commonly used to assess spontaneous locomotion and anxiety-like behavior (center avoidance / wall-hugging = thigmotaxis) in a large novel arena [3].

Indices calculated: latency to first enter, entries into, and time in the center area / total distance and mean speed / freezing duration and count

Square tank



Circular tank



* Zones and grids can be defined to match the tank shape (Square / Circular).

[1] Egan RJ, et al. Behav Brain Res. 2009;205:38–44. doi:10.1016/j.bbr.2009.06.022 [2] Cachat J, et al. Nat Protoc. 2010;5:1786–1799. doi:10.1038/nprot.2010.140
[3] Champagne DL, et al. Behav Brain Res. 2010;214:332–342. doi:10.1016/j.bbr.2010.06.001

Main Features

File I/O

- Load coordinate files (one fish: CSV or .txt / multiple fish: .txt only)
- Demo CSVs (per experiment type)
- Download Output_List.csv / per-frame CSV

Basic Settings

- Frame Rate (fps)
- Scaling Factor (mm/pix)
- Outlier Threshold /
  Long-term Missing Data Threshold
- Zone Duration Threshold / Zone Definition

Smoothing / Freezing

- Smoothing ON/OFF
- Smoothing Window / Gaussian Sigma
- Instantaneous Rate of Freezing Exclusion (mm/s) / Freeze Exclusion Duration (sec)

Tank Calibration

- [Square] Left/Right/Top/Bottom (pix) + Degree
- [Circular] Center X / Y and Diameter
- Grid Divisions [Square] X × Y (max 200 × 200)
- Grid Divisions [Circular] Radial × Angular

Analysis Duration

- Auto-set on file load; adjust with slider
- Manual entry of Start Time / End Time (min:sec)
- Analysis window of up to 150 min

Output & History

- List of analysis results
- Select all / download individual CSVs
- Analysis history retained
- On-screen heatmap / trajectory

Input Specifications (CSV Format)

Item	Description
File format	One fish: CSV or .txt; multiple fish: .txt only (no CSV). Max 20 MB
Character encoding	UTF-8 (Shift_JIS also works if the file has only numbers and delimiters)
Multiple fish (.txt)	Three columns per fish (X, Y, ProbId) side by side, tab- or comma-separated. Enter NaN where ProbId is missing
Required columns	X, Y, ProbId per fish (frames follow row order)
Coordinate system	Pixels (converted to mm with Scaling Factor [mm/pix])
Frame Rate	Set separately by the user (e.g., 30 fps)
Missing values	NaN / blank → short gaps are linearly interpolated; gaps lasting at least the Long-term Missing Data Threshold are treated as Long-term Missing and not included in the valid time
Demo CSV	One per experiment type; downloadable in the app

Parameter Details

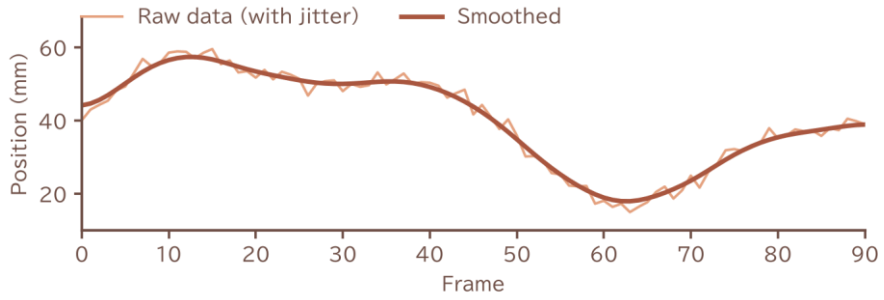
* See the Glossary at the end of this deck for term definitions.

Parameter	Unit	Description
Frame Rate	fps	Video frame rate; basis for time conversion. (Default: 30 fps)
Outlier Threshold	mm	Displacement above which a movement is treated as an outlier. (Default: 80 mm)
Long-term Missing Data Threshold	frame	Number of consecutive frames judged as long-term missing. (Default: 30 frames)
Zone Duration Threshold	frame or sec	Minimum stay needed to confirm an entry or exit. When set in seconds, seconds × fps is rounded to the nearest frame. (Default: 30 frames)
Scaling Factor	mm/pix	Converts pixels to real distance (mm). To relate real lengths to pixel lengths, measure an object of known length (e.g., the tank width) and enter its real length (mm) and image length (px) in the Scale fields; the factor is calculated automatically (average of up to two pairs).
Smoothing Window	frame or sec	Upper limit of the smoothing range. (Default: 1.0 sec)
Gaussian Sigma	—	Spread of the weights = smoothing strength. (Default: 1)
Instantaneous Rate of Freezing Exclusion	mm/s	Speed limit for freezing: at or below this Instantaneous Rate, the fish is stationary. (Default: 10 mm/s, following [6])
Freeze Exclusion Duration	sec	Freezing = Instantaneous Rate at or below the threshold for at least this long. (Default: 1.2 s)

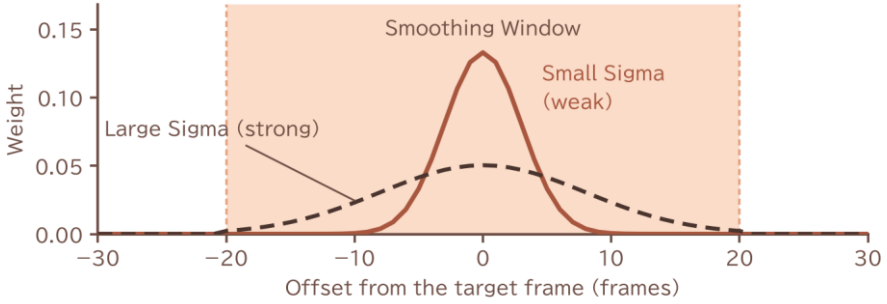
[6] Shinkai T, et al. Front Behav Neurosci. 2025;19:1624277. doi:10.3389/fnbeh.2025.1624277

Supplement: How Smoothing Works

① Effect of smoothing (simulated data)



② Weights applied to nearby frames (each sums to 1)



Why is it needed?

- Tracking data contain small frame-to-frame jitter (noise) even when the fish is not moving [6]
- Accumulated jitter inflates the calculated distance and speed [7]

How it works (Gaussian filter)

Each frame's position is replaced by a weighted average of neighboring frames. Weights are bell-shaped (Fig. ②): closer frames weigh more, and the weights always sum to 1. Unlike a simple moving average (equal weights), nearby frames count more, preserving the shape of the real movement. Applied separately to x and y after conversion to mm. Also used in fish swimming-trajectory analysis [4, 5].

Two parameters

- **Smoothing Window:** maximum range of frames used; frames outside are never used (dotted line in Fig. ②).
- **Gaussian Sigma: width of the bell. Small: concentrated nearby (weak smoothing); large: spread out (strong smoothing)**

→ Smoothing strength is set by Gaussian Sigma alone.

Cautions

Too much smoothing can mask meaningful behavioral signals [6]. Default is OFF. Report the settings you used in your paper.

[4] Dunn TW, et al. eLife. 2016;5:e12741. doi:10.7554/eLife.12741 [5] Heras FJH, et al. PLoS Comput Biol. 2019;15:e1007354. doi:10.1371/journal.pcbi.1007354
[6] Shinkai T, et al. Front Behav Neurosci. 2025;19:1624277. doi:10.3389/fnbeh.2025.1624277 [7] Ranacher P, et al. Int J Geogr Inf Sci. 2016;30:316–333. doi:10.1080/13658816.2015.1086924

Supplement: Processing Flow and Freezing Detection

① **Detect outliers and** fill short gaps → ② **Smoothing** (when ON) → ③ **Freezing detection** (Instantaneous Rate) → ④ **Long-term missing** segments overwritten last (missing)

* Long-term missing segments, and the first and last round(fps÷6) frames, are excluded from freezing detection

How freezing is detected

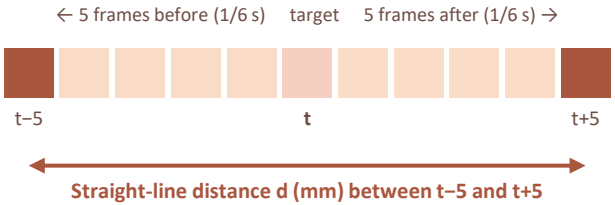
- For each frame, measure the straight-line distance between the points round(fps÷6) frames before and after it (5 frames = 1/6 s at 30 fps)
- Distance ÷ time (1/3 s) = **Instantaneous Rate** (mm/s)
- If the Instantaneous Rate stays at or below **Instantaneous Rate of Freezing Exclusion** for at least **Freeze Exclusion Duration**, it is counted as Freezing

* Defaults: 10 mm/s and 1.2 s (speed criterion follows [6])

* This Freezing detection is a simplified implementation of the method in [6] and does not reproduce that paper's classification

* Freezing is detected only when Smoothing is ON

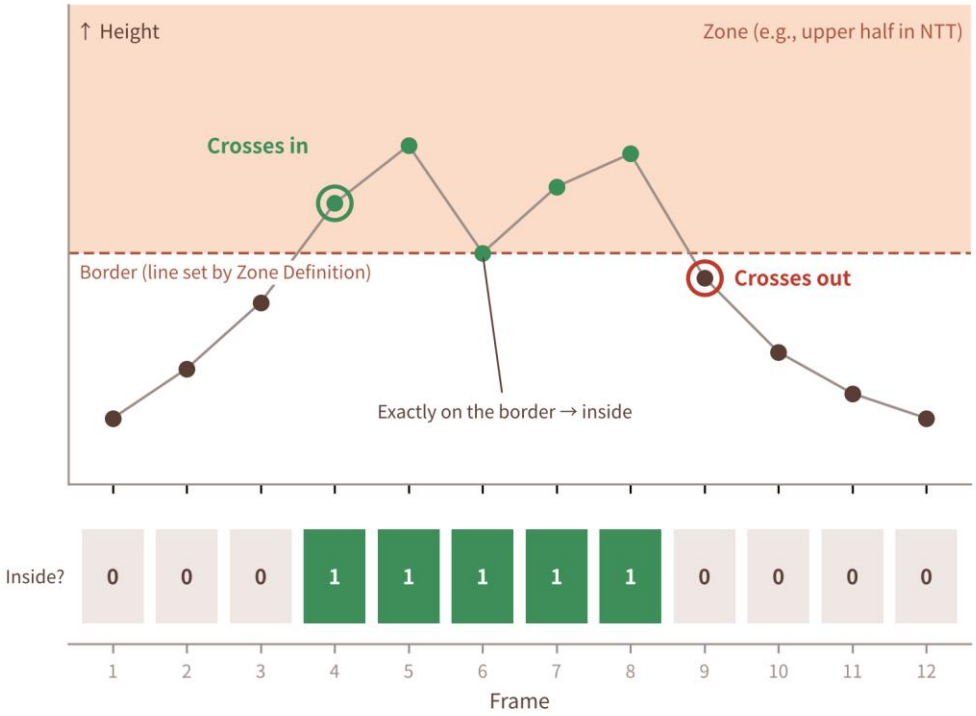
Calculating the Instantaneous Rate (30 fps example)



Instantaneous Rate = d ÷ (10 frames ÷ fps)

At 30 fps: d ÷ 1/3 s = d × 3 (mm/s)

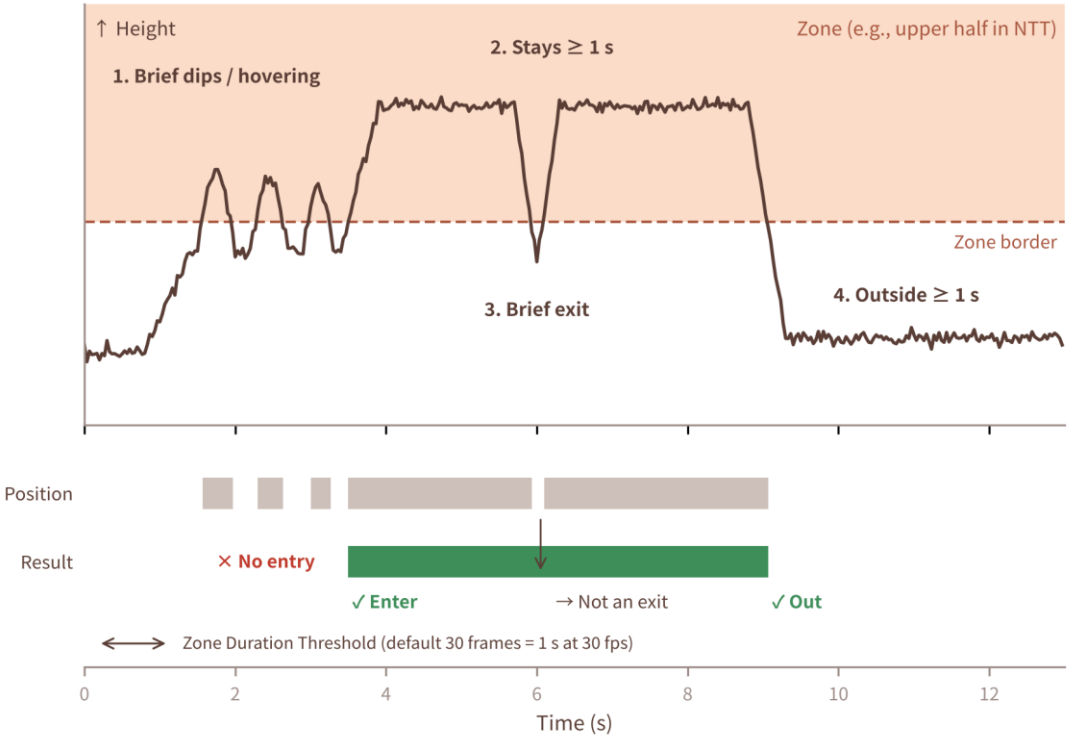
Supplement: Zone Detection (Enter / Out)



How Enter / Out are determined

- Each frame is checked against the border set by Zone Definition (in NTT with 1/2, the midline between Top and Bottom) to decide whether the fish is inside or outside the zone
- A position exactly on the border counts as inside
- Crossing into the zone alone is not an Enter; Enter is recorded when the fish stays in the zone for at least the Zone Duration Threshold
- Leaving works the same way: Out is recorded when the fish stays outside for at least the Zone Duration Threshold (next slide)
- OFT works the same way, based on whether the fish is inside the center area
- Enter / Out can be checked in the Enter/Out column of the per-frame CSV and as rings on the trajectory plot

Supplement: Zone Duration Threshold (Enter / Out)



How Zone Duration Threshold works

- Both when entering and when leaving, the state changes only after the fish stays on the same side for at least the Zone Duration Threshold
- Entering: brief dips into the zone and hovering at the border are not entries (1); staying 1 s or longer is an Enter (2)
- Leaving: a brief exit while in the zone is not an exit (3); staying outside 1 s or longer is an Out (4)
- Prevents tracking jitter and small movements at the border from distorting the number of entries and related outputs
- Set in frames or seconds (seconds × fps, rounded). Default: 30 frames (1 s at 30 fps, following [6])
- Main outputs affected: Number of Entries, Second Validated Entry, etc.

[6] Shinkai T, et al. Front Behav Neurosci. 2025;19:1624277. doi:10.3389/fnbeh.2025.1624277

Output Specifications (Output_List.csv Columns)

Column	Unit	Description
Name	—	Output file name (multiple fish: branch number appended)
Latency to Enter the Upper Half / Center Area	sec	Time to the first entry. Column name: Upper Half for NTT, Center Area for OFT. When NTT and OFT are exported in one list, both are shown as Upper Half (Center Area)
Second Validated Entry	sec	Time of the second validated entry; an entry is validated when the fish stays for at least the Zone Duration Threshold
Total Analysis	sec	Total length of the analysis window
Valid Duration	sec	Valid analysis time (long-term missing time subtracted)
Entry Duration	sec	Cumulative time spent in the zone
Number of Entries	count	Number of entries into the zone
Long-term Missing Data	frame	Total number of long-term missing frames. Outliers inside a long-term gap are counted; interpolated outliers are not
Long Gap in Time	frame	Frames of long-term missing data that overlap a zone visit
Total Distance	mm	Total distance traveled within the analysis window; long-term missing intervals are excluded. Equals the last Acm. Distance value in the per-frame CSV
Average Velocity	mm/s	Mean movement speed
Freezing Seconds	sec	Total freezing time
Freezing Count	count	Number of freezing events
Freezing Mean Duration	sec	Mean duration of freezing events
Active Phase Velocity	mm/s	Mean speed during non-freezing periods
Tank Shape	—	Tank shape (Square / Circular)
Created	—	Date and time of the analysis
* Freezing Seconds, Freezing Count, Freezing Mean Duration and Active Phase Velocity are calculated only when Smoothing is ON		

Output Specifications (Per-frame CSV Columns)

Column	Unit	Description
* The file opens with the same settings record as Output_List.csv. File name: <output file name>.csv		
Frame・Time	— / sec	Frame number and elapsed time from the start of the analysis
X Pix・Y Pix	pix	Raw coordinates as read from the input file
Revised X Axis・Revised Y Axis	mm	Coordinates after Degree correction and scaling
X Movement・Y Movement	mm	Per-axis displacement from the previous frame
Distance・Acm. Distance	mm	Displacement from the previous frame, and its running total (final row = total distance)
Speed・X Velocity・Y Velocity	mm/s	Speed and its per-axis components
Acceleration・X / Y Acceleration	mm/s²	Acceleration and its per-axis components
Cos Theta・Sin Theta	—	Cosine and sine of the heading
Movement Direction (Left/Right)	—	Whether the fish moved left or right
Movement Angle・Turning Angle	rad	Heading angle, and the change in heading from the previous frame
X Grid・Y Grid	—	Grid cell index. Grid Radial・Grid Angular for circular tanks
In Event Area・Enter/Out	—	Whether the fish is inside the target zone / entry and exit events (see slide 9)
Accumulated Duration in Event Area	sec	Cumulative time spent in the zone
Is Freezing	—	1 = Freezing, 0 = otherwise. Written only when Smoothing is ON

Notes on Use

Environment

- No installation needed. Open in your browser: https://bio-fish-models.com/
- Tested browsers: Google Chrome, Microsoft Edge, Mozilla Firefox and Safari (latest desktop versions)
- Communication is encrypted with HTTPS (HTTP is redirected to HTTPS).

Handling of uploaded data

- Data are stored on the server only temporarily for analysis (about 24 hours).
- No coordinate data or results are saved on your device (only a session-management cookie is stored).
- Download Output_List.csv and the per-frame CSV to keep your results.

Limitations

Current limitations

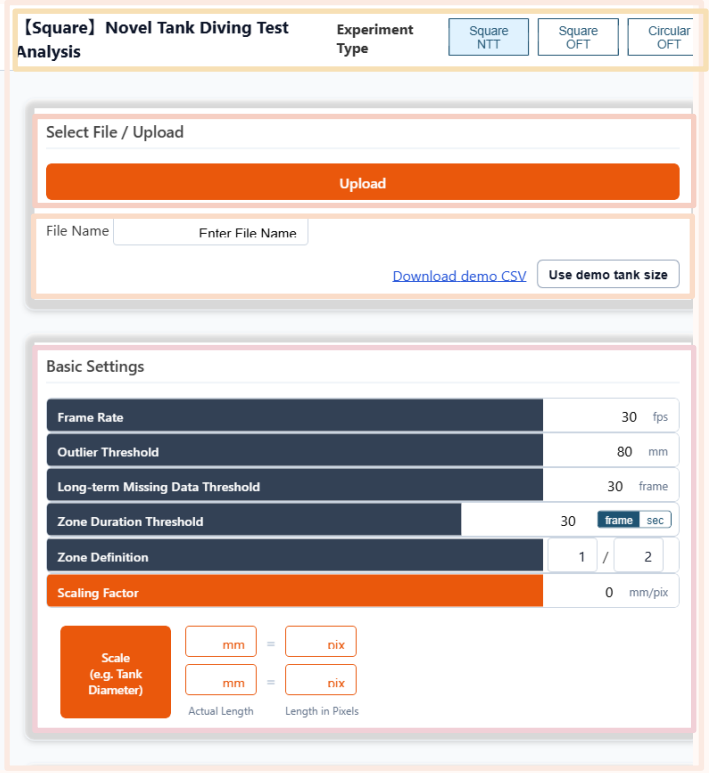
- Smartphone display is not tested
- Processing speed for large CSV files is untested
- Heatmaps and trajectories of multiple fish are shown one at a time (switch with View)

APPENDIX

UI Screen Guide

What to enter where on the screen — the role of each area and related specifications

① Experiment type / ②③ File input / ④ Basic Settings



What to enter

1 Select experiment type

Switch between Square NTT / Square OFT / Circular OFT. The following input fields and analysis logic change accordingly.

2 Upload coordinate file

Select the time-series file of x, y coordinates exported by your tracking software: CSV or .txt for one fish, .txt only for multiple fish (CSV not accepted).

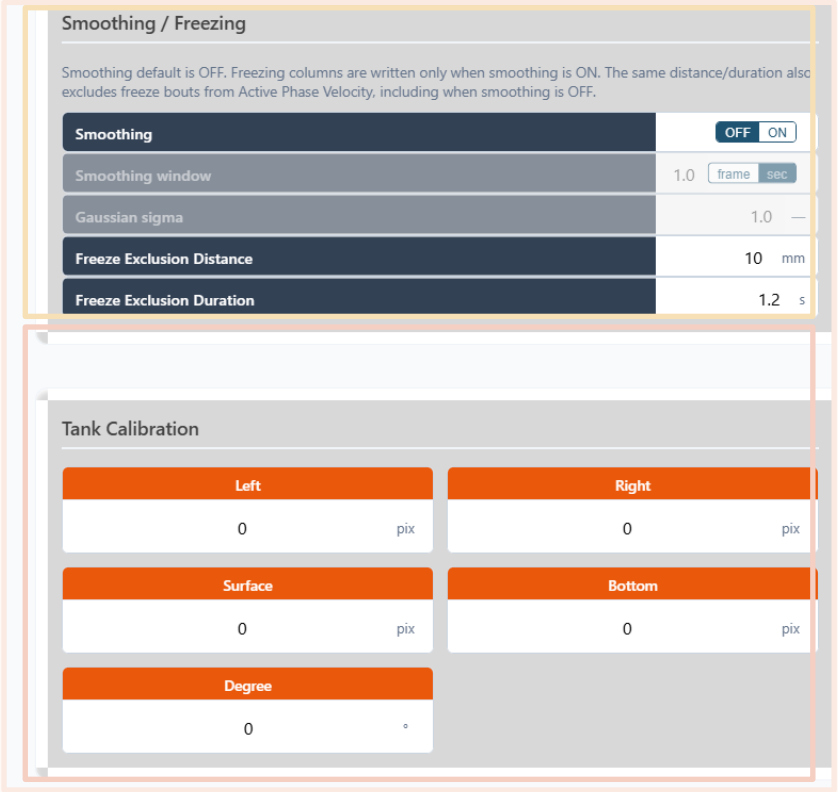
3 Demo CSV / Use demo tank size

Get a sample CSV and apply the default tank size in one click.

4 Basic Settings

Set Frame Rate, Outlier Threshold, Long-term Missing Data Threshold, Zone Duration Threshold (frames or seconds), Zone Definition and Scaling Factor (mm/pix).

⑤ Smoothing / Freezing / ⑥ Tank Calibration



What to enter

5 Smoothing / Freezing

When Smoothing is ON, small tracking jitter (noise) is smoothed before speed and other values are calculated (default OFF).

- **Smoothing Window: upper limit of the frame range (frames or seconds)**
- **Gaussian Sigma: spread of the weights = smoothing strength**

Instantaneous Rate of Freezing Exclusion (mm/s) and Freeze Exclusion Duration (sec) are also set here.

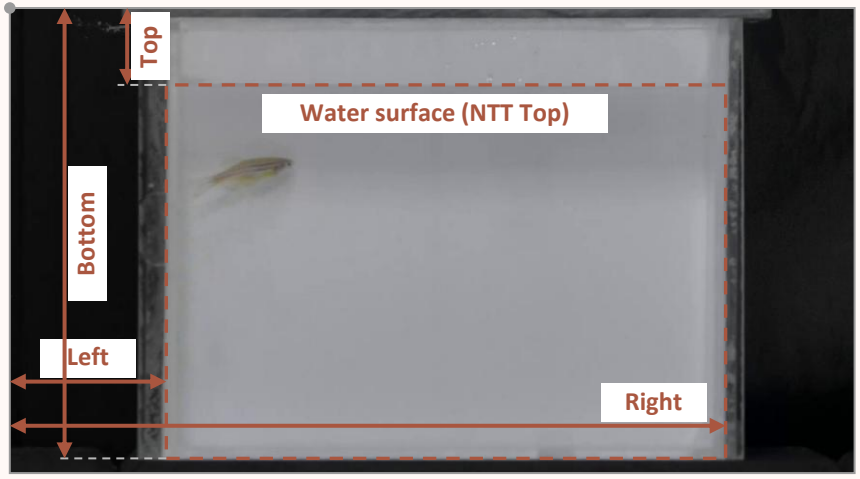
6 Tank Calibration

Square: Left / Right / Top / Bottom (pix) and Degree (tilt).
Circular: Center X / Center Y / Diameter (pix).

Tank Calibration: What to Measure (Diagram)

Square tank

(0, 0)

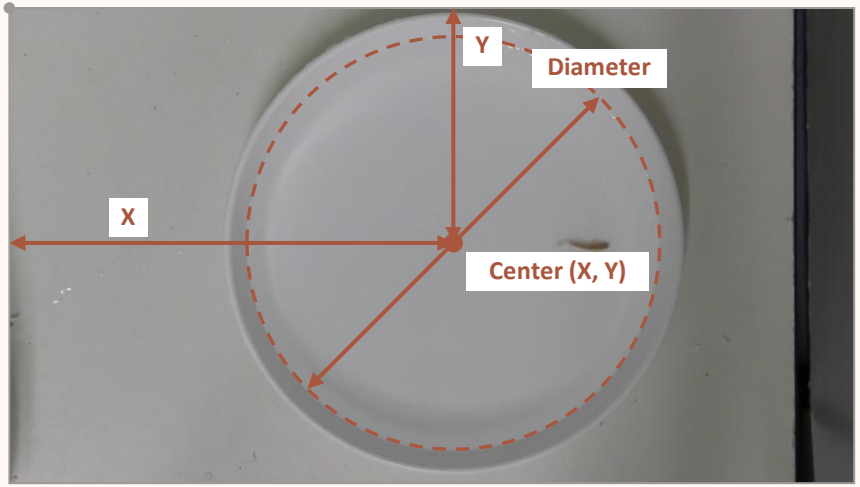


Frame (video image)

* Left / Right: measured at the tank bottom; Top / Bottom: at the tank's left edge

Circular tank

(0, 0)



Frame (video image)

* Dashed line = inner edge of the tank; measure Center and Diameter on this edge

Tank Calibration: What to Measure (Definitions)

Item	Definition
Square tank	
Left	Distance from the left edge of the frame to the left edge of the tank (at the tank bottom)
Right	Distance from the left edge of the frame to the right edge of the tank (at the tank bottom)
Top	Distance from the frame top to the water surface (NTT) or tank top (OFT), at the tank's left edge
Bottom	Distance from the top edge of the frame to the bottom edge of the tank (at the tank's left edge)
Degree	Tilt correction (45–135°; none = 90°). >90°: clockwise; <90°: counterclockwise; pivot: tank's lower-left corner
Circular tank	
Center	X and Y coordinates of the center of the tank
Diameter	Diameter of the tank

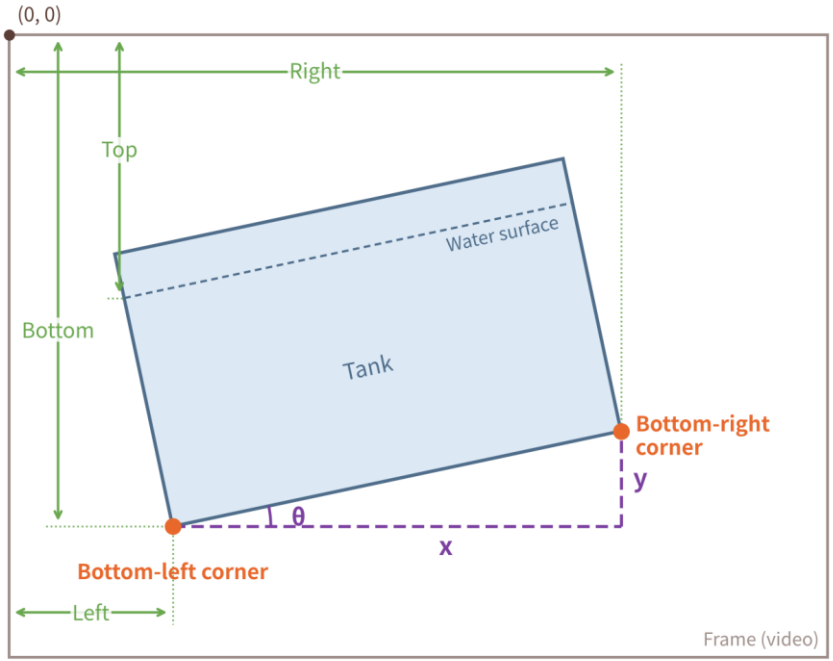
Unit: pixels (px) / Origin: top-left corner of the frame (0, 0) / Degree: blank or 0 is treated as 90°

How to measure in ImageJ (Fiji) (recommended)

1. Open a single frame of the video in ImageJ. ImageJ can open AVI videos only (uncompressed or MJPEG). Convert MP4, MOV, etc. to AVI, or save one frame as an image (PNG/JPEG) and open that.
2. Select the Straight Line tool and draw a line from the edge of the frame to the point you want to measure (hold Shift to keep the line horizontal or vertical).
3. Press Ctrl + M (Analyze > Measure). The length in pixels appears in the Length column of the Results window.

* If a scale has been set (Analyze > Set Scale), the result will not be in pixels. Keep the scale unset (pixels).

Tank Calibration: Measuring a Tilted Tank

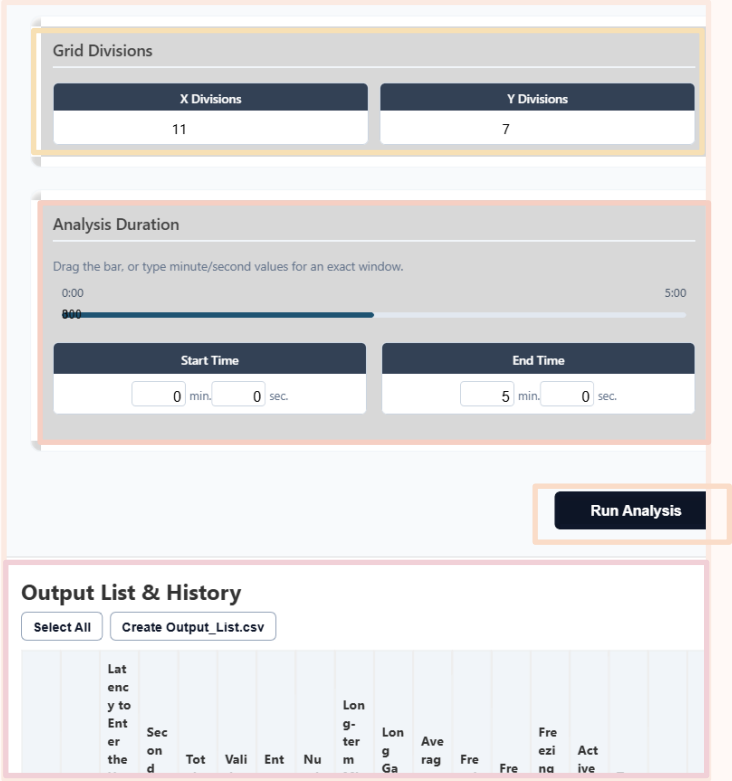


When the tank is tilted relative to the frame

1. With the ImageJ Point tool, click the bottom-left and bottom-right corners of the tank and press Ctrl + M to record their coordinates (X, Y)
2. Enter the values as follows
   - Left: X of the bottom-left corner / Bottom: Y of the bottom-left corner
   - Right: X of the bottom-right corner
   - Top: from the frame top to the water surface (NTT) or tank top (OFT)
3. Calculate the tilt θ
   - x = X of bottom-right corner − X of bottom-left corner
   - y = Y of bottom-left corner − Y of bottom-right corner
   - Excel: θ = DEGREES(ATAN2(x, y))
4. Enter 90 + θ in Degree (θ is positive if the right side is higher, negative if lower)

* Drawing a Straight Line along the bottom edge from the bottom-left to the bottom-right corner and pressing Ctrl + M shows θ directly in the Angle column of Results

⑦⑧ Analysis settings / ⑨ Run / ⑩ Output



What to enter

7 Grid Divisions

Square: X Divisions × Y Divisions (max 200 × 200)
Circular: Radial Divisions × Angular Divisions
Used as units for the time-in-zone map and entry counts.

8 Analysis Duration

Set automatically on file load (end time rounded up, e.g., 3.3 s → 4 s). Adjust with the slider or Start / End Time (min:sec); max 150 min.

9 Run Analysis

Confirms the settings and runs the analysis; results are added to the list below.

10 Output list / Save CSV

Shows results for each fish. With multiple fish, a suffix is added to Name.
Download Output_List.csv and the per-frame CSV.

Glossary (1/2) — Tests, Input Data and Preprocessing

Term	Definition
NTT	Novel Tank Diving Test. Observes zebrafish behavior after transfer to a novel tank; commonly used to assess anxiety-like behavior.
OFT	Open Field Test. Observes behavior in a large arena; commonly used to assess spontaneous locomotion and anxiety-like behavior.
ProbId	Column identifying each fish in the input file. Three columns per fish (X, Y, ProbId) side by side, numbered per fish (X1, Y1, ProbId1 …). Enter NaN where the value is missing.
Scaling Factor	Factor converting pixels to real length in mm (mm/pix), obtained by relating the real length of an object of known size to its length in the image.
Degree	Tilt-correction angle set in Tank Calibration (45–135°; none = 90°). Coordinates are rotated by this angle before conversion to mm.
Outlier	A frame whose displacement from the previous frame exceeds the Outlier Threshold. Treated as a tracking error and replaced with missing data (NaN); like other gaps, short gaps are interpolated and only gaps lasting at least the Long-term Missing Data Threshold are treated as long-term missing.
Long-term Missing	A segment in which missing frames (including outliers) continue for at least the Long-term Missing Data Threshold; not included in the valid time.
Smoothing	Processing that reduces noise in coordinate data, using a Gaussian filter.

Glossary (2/2) — Analysis Metrics and Output

Term	Definition
Instantaneous Rate	Instantaneous speed (mm/s): straight-line distance between the points round(fps÷6) frames before and after the frame (at 30 fps, t−5 and t+5 — 10 frames = 1/3 s apart) divided by the elapsed time. The value itself is not written to the CSV.
Freezing	Near-immobile state. Periods in which the Instantaneous Rate stays at or below the threshold for at least the set duration are counted as Freezing. Calculated only when Smoothing is ON.
Active Phase Velocity	Mean movement speed excluding freezing periods (mm/s).
Zone (In Event Area)	Target region of the analysis: the upper half for NTT, the center area for OFT. Written to the per-frame CSV as the In Event Area column (1 = inside, 0 = outside).
Zone Duration Threshold	Minimum stay needed to confirm an entry or exit. The state changes only when the fish stays inside or outside the zone for at least this time, so brief entries or exits and hovering at the border are not counted. When set in seconds, seconds × fps is rounded to the nearest frame (default: 30 frames).
Grid	Unit of spatial aggregation dividing the tank into cells: X Divisions × Y Divisions (Square), Radial × Angular (Circular). The cell index of each frame is written to the per-frame CSV (X Grid, Y Grid).
Valid Duration	Valid analysis time: the total analysis window (Total Analysis) minus the long-term missing frames divided by fps.
Second Validated Entry	Time of the second validated entry; an entry is validated when the fish stays for at least the Zone Duration Threshold. Blank when there is no such entry.