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FreeClimber-FNG: Automated Detection of Failed Negative Geotaxis and Fall Distance in Drosophila Climbing Assays

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Software

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Summary

Negative geotaxis (climbing) assays in *Drosophila melanogaster* are widely used to classify motor performance and subsequent disruption by age, traumatic brain injury, genetic perturbation, or pharmacological manipulation. The open-source *FreeClimber* platform automates extraction of climbing velocity from video recordings using background subtraction, particle tracking, and local linear regression on vial-wise mean vertical position traces of *Drosophila melanogaster* (Spierer et al., 2021).

Investigators can additionally score failed negative geotaxis (FNG) events. FNG is defined as an event in which a fly climbs but then loses footing and falls within the assay window. FNG is typically hand-scored, which is time-consuming, rater-dependent, and difficult to reproduce.

FreeClimber-FNG is a Python extension to *FreeClimber* that adds automated detection of FNG events and computation of fall distance using the same frame-resolved position data already produced by *FreeClimber*. The extension runs automatically during the standard pipeline, requires no GUI changes, and yields machine-readable `.fng.csv` output suitable for statistical analysis. This tool provides a reproducible, scriptable alternative to manual FNG scoring.

Statement of Need

Drosophila climbing assays remain a central behavioral measure in neuroscience and aging research due to their simplicity, experimental flexibility, and sensitivity to subtle motor impairments (Gargano et al., 2005). Laboratories may choose to report the presence of FNG events in addition to the standard velocity / height metrics, as falls may characterize motor discoordination, intoxication, fatigue, or insult related deficit. However:

1. No commonly used open-source tool automates FNG detection
2. Most laboratories rely on manual review of videos
3. Scoring is difficult to standardize across raters
4. Results are not easily auditable or reproducible

FreeClimber-FNG addresses this gap by integrating directly with *FreeClimber's* existing data pipeline and by adding an analysis layer for identifying climb–fall sequences and quantifying fall distance. Researchers who already use *FreeClimber* can therefore obtain both velocity / height and fall-behavior measures from the same recorded video without additional steps.

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Research Impact Statement

Automated detection of failed negative geotaxis events enables researchers to quantify motor instability and recovery behavior in Drosophila assays without manual video scoring. By providing standardized, machine-readable outputs for fall events and fall distance, FreeClimber-FNG improves reproducibility and scalability in behavioral analysis workflows. This extension supports ongoing and future research into aging, neurodegeneration, traumatic brain injury, and pharmacological effects on locomotor coordination.

Software Design

FreeClimber-FNG is designed as a modular extension to the existing FreeClimber analysis pipeline. The software reuses FreeClimber's established video processing, particle detection, and filtering stages, and introduces additional analysis logic that operates on the filtered, per-frame position data. This design minimizes duplication, preserves backward compatibility, and allows users to obtain both climbing velocity and fall-related metrics from the same tracked data without modifying upstream components.

Software Description

Integration with *FreeClimber*

FreeClimber-FNG is implemented as an addition to the existing `detector` class in `detector.py`. The core *FreeClimber* workflow remains unchanged:

1. **Video → cleaned frame stack**
   Background subtraction and cropping.
2. **Frame stack → detected fly positions**
   Particle detection with TrackPy and filtering.
3. **Filtered positions → vial-wise height traces**
   Mean y-position per frame for each vial.
4. **Height traces → FNG events (this extension)**
   Detection of peaks and subsequent descents.

The extension operates entirely on the `df_filtered` table produced in `step_5`, requiring only:

- `frame`
- `vial`
- y (after FreeClimber's inversion, higher y = higher in vial)

The extension writes a standardized per-event table to: "videoname.fng.csv"

FNG Detection Logic

For each vial:

1. Compute the mean vertical position for each frame.
2. Apply a centered rolling-mean smoothing kernel.
3. Normalize the trace to a 0–1 range.
4. Detect *climb peaks*.
5. Identify the subsequent fall segment and compute:

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- `frame_peak`
- `frame_fall_start`
- `frame_fall_end`
- normalized rise magnitude
- normalized drop magnitude
- fall distance in pixels
- fall distance in centimeters (if pixel scaling available)

All parameters (smoothing window, minimum rise, minimum drop, minimum separation) are accessible for tuning, enabling adaptation to different rigs and frame rates.

Implementation

The tool is written in Python and uses the same scientific stack as *FreeClimber*:

- **NumPy**, **pandas** for data manipulation
- **SciPy** (`find_peaks`) for peak detection
- **TrackPy** for object tracking
- **Matplotlib** for optional visualization

The extension does not modify the GUI or underlying detection logic, ensuring backward compatibility. The FNG routines can also be imported independently and run on any dataset containing per-frame vial height traces.

Quality Control

The FNG detection module was validated on climbing videos ($n = 120$) from laboratory produced negative geotaxis assays. During development, event detections were cross-checked with the underlying height traces and corresponding video frames to ensure consistency between hand raters and the software.

Thresholds, smoothing windows, and event-gap parameters were tuned until automated detections matched researcher-verified climb–fall behavior. As assay geometry and frame rate vary across laboratories, the software exposes these parameters for user control. Recommended practice is to:

1. Run *FreeClimber* on a small subset of videos.
2. Inspect detected FNG events alongside height traces.
3. Adjust parameters as needed.
4. Apply the final configuration batch-wise across experiments.

Availability

- **Source Code:** https://github.com/jordanvasu/FreeClimber-FNG-Adaptation-Vasu-2025-
- **License:** MIT
- **Operating Systems:** Any system supported by FreeClimber (Windows, macOS, Linux).
- **Dependencies:** Python 3.6

This version of the software is archived on Zenodo: https://doi.org/10.5281/zenodo.17577324

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AI Usage Disclosure

The software was developed and validated by the authors. General-purpose development tools were occasionally used to assist with debugging or refactoring during development. The algorithm design, parameter selection, validation against experimental data, and integration into the FreeClimber pipeline were performed by the research team.

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